

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

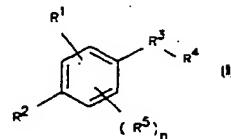
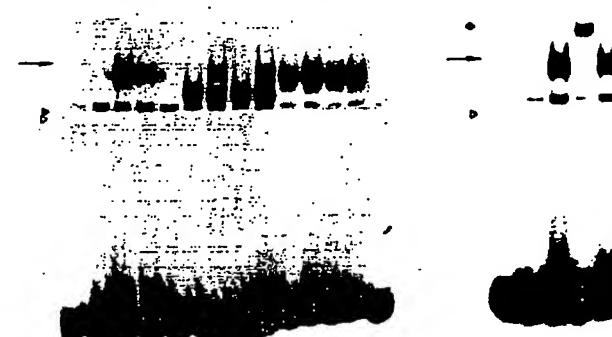


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|  |  |  |  |
|--|--|--|--|
| (51) International Patent Classification <sup>5</sup> :<br><b>G01N 33/566, 33/50, C07K 14/705, C07D 339/08, 339/06, 327/04, 319/06, 317/30, 409/04, 333/38, C07C 63/49, 63/66, 65/36, A61K 31/335, 31/19</b>   |  | A2   | (11) International Publication Number: <b>WO 94/12880</b>          |
|  |  |  | (43) International Publication Date: <b>9 June 1994 (09.06.94)</b> |
| (21) International Application Number: <b>PCT/US93/11492</b>   |  | (74) Agents: <b>PERRYMAN, David, G. et al.; Needle &amp; Rosenberg, 127 Peachtree Street, N.E., Suite 1200, Atlanta, GA 30303 (US).</b>  |  |
| (22) International Filing Date: <b>24 November 1993 (24.11.93)</b>   |  | (81) Designated States: <b>AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</b> |  |
| (30) Priority Data:<br><b>07/982,174 25 November 1992 (25.11.92) US<br/>07/982,305 25 November 1992 (25.11.92) US</b>  |  | Published<br><i>Without international search report and to be republished upon receipt of that report.</i>   |  |
| (71) Applicants: <b>LA JOLLA CANCER RESEARCH FOUNDATION [US/US]; 10901 North Torrey Pines Road, La Jolla, CA 92037 (US). SRI INTERNATIONAL [US/US]; 333 Ravenswood Avenue, Menlo Park, CA 94025 (US).</b>  |  |  |  |
| (72) Inventors: <b>PFAHL, Magnus; 605 North Rios Avenue, Solana Beach, CA 92075 (US). ZHANG, Xiao-kun; 9176F Regents Road, La Jolla, CA 92037 (US). LEHMANN, Jurgen, M.; 773 S. Nardo, Apartment H-1, Solana Beach, CA 92075 (US). DAWSON, Marcia, L; P.O. Box 1033, Menlo Park, CA 94026 (US). CAMERION, James, F.; 881 Linda Vista, P.O. Box 69, Moss Beach, CA 94038 (US). HOBBS, Peter, D.; 2176 Williams Street, Palo Alto, CA 94306 (US). JONG, Ling; 1166 Sunnyvale-Saratoga Road, Apartment #22, Sunnyvale, CA 94087 (US).</b> |  |  |  |
| (54) Title: <b>RXR HOMODIMER FORMATION AND BRIDGED BICYCLIC AROMATIC COMPOUNDS AND THEIR USE IN MODULATING GENE EXPRESSION</b>   |  |  |  |

RECEPTOR: **RXR      RXR+TR      RXR+RAR      RAR**  
LIGAND: **- - 9-cis RA      T3 - 9-cis T3      T3+9cis - 9-cis RA      RA+9-cis 9-cis**

Ab: **— — — αF NI**  
**9-cis: — + + +**  
**F-RXR: — + + +**



**BEST AVAILABLE COPY**

(57) Abstract

The invention provides a method of screening a substance for the ability to affect the formation of a retinoid X receptor homodimer comprising combining the substance and a solution containing retinoid X receptors and determining the presence of homodimer formation. The screening method can be used to determine compounds which selectively activate homodimer formation and heterodimer formation. Also provided is a method of screening a substance for an effect on a retinoid X receptor homodimer's ability to bind DNA comprising combining the substance with the homodimer and determining the effect of the compound on the homodimer's ability to bind DNA. Finally, the invention provides methods of activating retinoid X receptor homodimer formation. Bridged bicyclic aromatic compounds are provided having structure (I); wherein R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and n are as defined herein. The compounds are useful for modulating gene expression of retinoic acid receptors, vitamin D receptors and thyroid receptors. Pharmaceutical compositions and methods for modulating gene expression are provided as well.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

|    |                          |    |  |    |                          |
|----|--------------------------|----|--|----|--------------------------|
| AT | Austria                  | GB | United Kingdom                           | MR | Mauritania               |
| AU | Australia                | GE | Georgia                                  | MW | Malawi                   |
| BB | Barbados                 | GN | Guinea                                   | NE | Niger                    |
| BE | Belgium                  | GR | Greece                                   | NL | Netherlands              |
| BF | Burkina Faso             | HU | Hungary                                  | NO | Norway                   |
| BG | Bulgaria                 | IE | Ireland                                  | NZ | New Zealand              |
| BJ | Benin                    | IT | Italy                                    | PL | Poland                   |
| BR | Brazil                   | JP | Japan                                    | PT | Portugal                 |
| BY | Belarus                  | KE | Kenya                                    | RO | Romania                  |
| CA | Canada                   | KG | Kyrgyzstan                               | RU | Russian Federation       |
| CF | Central African Republic | KP | Democratic People's Republic<br>of Korea | SD | Sudan                    |
| CG | Congo                    | KR | Republic of Korea                        | SE | Sweden                   |
| CH | Switzerland              | KZ | Kazakhstan                               | SI | Slovenia                 |
| CI | Côte d'Ivoire            | LI | Liechtenstein                            | SK | Slovakia                 |
| CM | Cameroon                 | LK | Sri Lanka                                | SN | Senegal                  |
| CN | China                    | LU | Luxembourg                               | TD | Chad                     |
| CS | Czechoslovakia           | LV | Latvia                                   | TG | Togo                     |
| CZ | Czech Republic           | MC | Monaco                                   | TJ | Tajikistan               |
| DE | Germany                  | MD | Republic of Moldova                      | TT | Trinidad and Tobago      |
| DK | Denmark                  | MG | Madagascar                               | UA | Ukraine                  |
| ES | Spain                    | ML | Mali                                     | US | United States of America |
| FI | Finland                  | MN | Mongolia                                 | UZ | Uzbekistan               |
| FR | France                   |    |  | VN | Viet Nam                 |
| GA | Gabon                    |    |  |    |                          |

RXR HOMODIMER FORMATION AND  
BRIDGED BICYCLIC AROMATIC COMPOUNDS AND  
THEIR USE IN MODULATING GENE EXPRESSION

5

TECHNICAL FIELD

This invention relates generally to the regulation of gene expression by retinoid receptors, and more particularly relates to the retinoid X receptor and novel bridged bicyclic aromatic compounds that 10 are useful in modulating gene expression by retinoic acid receptors, retinoid X receptors, vitamin D receptors and thyroid receptors.

BACKGROUND ART

15 The vitamin A metabolite all-trans-retinoic acid (RA) and its natural and synthetic derivatives (retinoids) exert a broad range of biological effects<sup>1-2</sup>. Clinically, retinoids are important therapeutics in the treatment of skin diseases and cancers<sup>3-6</sup>. Understanding how the multitude of retinoid actions can be mediated at 20 the molecular level has been greatly enhanced by the cloning and characterization of specific nuclear receptors, the retinoic acid receptors (RARs)<sup>7-12</sup> and the retinoid X receptors (RXRs)<sup>13-17</sup>. RARs and RXRs are part of the steroid/thyroid hormone receptor superfamily<sup>18,19</sup>. Both types of receptors are encoded by three distinct genes,  $\alpha$ ,  $\beta$ , and 25  $\gamma$ , from which, in the case of RARs, multiple isoforms can be generated<sup>20-22</sup>. Interestingly, while RARs are specific to vertebrates, the RXRs have been well conserved from *Drosophila* to man<sup>17,23</sup>. Despite the considerable advances in the understanding of the molecular mechanisms of retinoid receptor action in recent years, a central 30 question of whether distinct molecular pathways for naturally occurring retinoids exist has not yet been answered. The recent observation that the RA stereoisomer 9-cis-RA binds with high affinity to RXRs<sup>23,24</sup> suggested a retinoid response pathway distinct from that of all-trans-RA. However, it was almost simultaneously discovered by 35 several laboratories that RARs require interaction with auxiliary receptors for effective DNA binding and function and that RXRs are such auxiliary receptors<sup>15,16,26-29</sup>. Hence, RARs appear to function

effectively only as heterodimeric RAR/RXR complexes, or in combination with comparable auxiliary proteins that still need to be identified.

Similarly, RXRs were shown to require RARs, thyroid hormone receptors (TRs), or Vitamin D<sub>3</sub> receptors (VDRs) for effective DNA binding<sup>15,16,26-29</sup>.

- 5 Thus, from these DNA binding studies, RXRs appeared to be able to function predominantly if not exclusively as auxiliary receptors, thereby playing a crucial role in generating a high degree of diversity and specificity of transcriptional controls and mediating the highly pleiotropic effects of different hormones by increasing DNA 10 affinity and specificity for at least 3 different classes of ligand-activated receptors.

Contrary to these findings, the present invention provides that RXRs form homodimers. The invention provides that these

- 15 homodimers effectively bind to specific response elements in the absence of auxiliary receptors and their DNA binding specificity is distinct from that of the RXR containing heterodimers. The invention demonstrates a novel mechanism for retinoid action by which a ligand induced-homodimer mediates a distinct retinoid response pathway.
- 20 Additionally, ligands are provided which selectively activate RXR homodimer formation.

The present invention also provides a new class of retinoids in the form of bridged bicyclic aromatic compounds as will be described in detail herein. These new compounds are useful for regulating and/or eliciting selective gene expression by receptors in the retinoic acid family, i.e., RARs, RXRs, vitamin D receptors (VDRs), and thyroid hormone receptors (THRs). While not wishing to be bound by theory, it is postulated that the presently disclosed and 30 claimed compounds are effective in modulating gene expression by virtue of their capability of interacting with a receptor protein that binds to the aforementioned receptors. The compounds are also believed to be useful in modulating gene expression by inducing formation of RXR homodimers in addition to RAR-RXR, VDR-RXR and THR-RXR heterodimers.

The novel compounds are thus useful for controlling cellular processes that are regulated by thyroid hormone, vitamin D, and retinoids such as 9-cis-retinoic acid, the natural ligand for RXR. Thus, acne, leukemia, psoriasis, and skin aging, all of which are 5 regulated by retinoic acid, may be treated using the compounds of the invention, as may bone calcification, regulated by vitamin D, and energy levels, regulated by thyroid hormone. Unlike the synthetic retinoids of the prior art, the present compounds are believed to provide for substantially reduced side effects and teratogenicity.

10

#### SUMMARY OF THE INVENTION

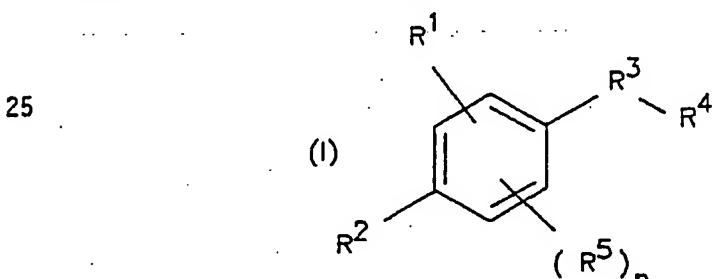
The invention provides a method of screening a substance for the ability to affect the formation of a retinoid X receptor 15 homodimer comprising combining the substance and a solution containing retinoid X receptors and determining the presence of homodimer formation. Also provided is a method of screening a substance for an effect on a retinoid X receptor homodimer's ability to bind DNA comprising combining the substance with the homodimer and determining 20 the effect of the compound on the homodimer's ability to bind DNA. A method of inhibiting an activity of a retinoid X receptor heterodimer comprising increasing the formation of a retinoid X receptor homodimer, thereby preventing the retinoid X receptor from forming a heterodimer and preventing the resulting heterodimer activity is also 25 provided. A method of inhibiting an activity of a retinoid X receptor homodimer is also provided. A method of determining an increased probability of a pathology associated with retinoid X receptor homodimer formation and treating such pathology are further provided. In addition, a method of screening a response element for binding with 30 a retinoid X receptor homodimer is provided. Finally, the invention provides methods of activating retinoid X receptor homodimer formation.

Additionally, it is a primary object of the invention to 35 address the above-mentioned need in the art by providing novel compounds useful to modulate gene expression by a receptor in the retinoic acid family of receptors. It is another object of the

invention to provide novel compounds useful to control cell differentiation processes regulated by thyroid hormone, vitamin D, and/or retinoids such as 9-cis-retinoic acid. It is still another object of the invention to provide novel compounds in the form of bridged, 5 bicyclic aromatic structures. It is yet another object of the invention to provide pharmaceutical compositions containing one or more of the novel compounds. It is a further object of the invention to provide a method for modulating gene expression of a receptor selected from the group consisting of retinoic acid receptors, 10 retinoid X receptors, vitamin D receptors, and thyroid receptors. It is still a further object of the invention to provide a method for controlling cell differentiation processes regulated by thyroid hormone, vitamin D, and/or retinoids such as 9-cis-retinoic acid.

15 Additional objects, advantages and novel features of the invention will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention.

20 In one aspect, then, the invention relates to novel compounds having the structural formula (I)



30 in which:

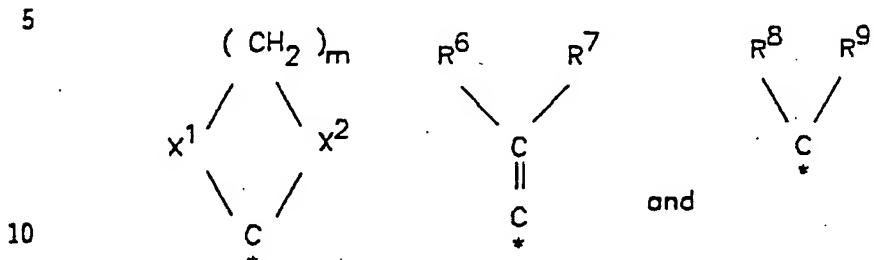
$R^1$  is selected from the group consisting of lower alkyl and adamantyl;

35  $R^2$  is  $-O-R^6$  or  $-S-R^6$  where  $R^6$  is lower alkyl; or where  $R^1$  is ortho to  $R^2$ ,  $R^1$  and  $R^2$  may be linked together to form a 5- or 6-membered cycloalkylene ring, either unsubstituted or substituted with 1 to 4 lower alkyl groups, and optionally containing

1 or 2 heterocyclic atoms selected from the group consisting of O, S and NR where R is hydrogen or lower alkyl, preferably adjacent to the aromatic ring;

R<sup>3</sup> is selected from the group consisting of carbonyl,

5

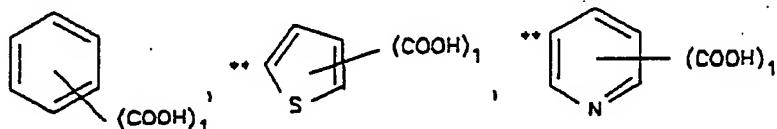


10

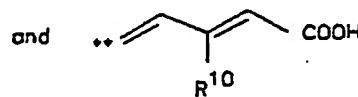
in which X<sup>1</sup> and X<sup>2</sup> are independently selected from the group consisting of O, S and methylene, wherein at least one of X<sup>1</sup> and X<sup>2</sup> is O or S, or 15 wherein one of X<sup>1</sup> and X<sup>2</sup> is NR, and the other is methylene, m is 2 or 3, R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup> and R<sup>9</sup> are independently hydrogen or lower alkyl, with the proviso that when n is 0, R<sup>6</sup> and R<sup>7</sup> are not both hydrogen and R<sup>8</sup> and R<sup>9</sup> are not both hydrogen, or R<sup>8</sup> and R<sup>9</sup> may be linked together to form a cycloalkylene ring containing 3 to 6 carbon atoms, and \* represents 20 the point of attachment of the R<sup>3</sup> substituent to the remainder of the molecule; and

R<sup>4</sup> is selected from the group consisting of

25



30



in which R<sup>10</sup> is hydrogen or methyl, 1 is 0 or 1, and \*\* represents the point of attachment of the R<sup>4</sup> substituent to the remainder of the 35 molecule,

the R<sup>5</sup> are independently selected from the group consisting of lower alkyl and lower alkoxy; and

$n$  is 0, 1, 2 or 3,  
with the proviso that if  $n$  is 0,  $R^3$  is other than  
carbonyl,  $*C=CH_2$  or  $CH_2$ .

5 The invention also encompasses pharmaceutically acceptable  
esters, amides and salts of such compounds, as will be explained in  
detail, infra.

10 In other aspects, the invention relates to pharmaceutical  
compositions containing the aforementioned compounds and to methods of  
using the compounds to modulate selective gene expression by a  
receptor in the retinoic acid family of receptors.

#### BRIEF DESCRIPTION OF THE DRAWINGS

15 Figure 1 shows 9-cis-retinoic acid induces RXR homodimer  
binding on TREpal.

20 (a) In vitro synthesized RXR $\alpha$  was incubated either with  
(+ or without (-) indicated hormones ( $10^{-7}$  M 9-cis-RA;  $10^{-6}$  M RA;  $10^{-6}$   
M T<sub>3</sub>) in the presence or absence of in vitro synthesized TR $\alpha$  or RARB  
for 30 min at room temperature. After this preincubation, the  
reaction mixtures were analyzed by gel retardation assay using  
<sup>32</sup>P-labeled TREpal as probe. Lane 1 represents the nonspecific binding  
25 of unprogrammed reticulocyte lysate. Open triangles indicate the  
nonspecific complex observed with unprogrammed reticulocyte lysate.  
Solid triangles indicate the specific TR $\alpha$ -RXR $\alpha$  heterodimer binding.  
Arrows indicate specific RXR $\alpha$  homodimer binding. The RXR $\alpha$ /RARB  
heterodimer migrates at the same position as the RXR $\alpha$  homodimer. For  
30 comparison, the effect of 9-cis-RA on RARB binding is shown.

25 (b) To determine that 9-cis-RA induced DNA binding  
complex contains RXR $\alpha$  protein, Flag-RXR $\alpha$  (F-RXR), an RXR $\alpha$  derivative  
that contains an eight-amino-acid epitope (Flag) at its amino terminal  
end which can be recognized by a specific anti-Flag monoclonal  
35 antibody, was constructed. In vitro synthesized F-RXR $\alpha$  was incubated  
either with (+) or without (-)  $10^{-7}$  M 9-cis-RA in the presence of

either specific anti-Flag antibody ( $\alpha F$ ) or nonspecific preimmune serum (NI) for 30 min at room temperature. The effect of anti-Flag antibody on F-RXR $\alpha$  binding in the presence of 9-cis-RA was then analyzed by gel retardation assay using  $^{32}P$ -labeled TREpal as a probe. Lane 1 represents the nonspecific binding of unprogrammed reticulocyte lysate (open triangles). Arrows indicate the specific F-RXR $\alpha$  homodimer and RAR-RXR heterodimer binding. Diamonds indicate the anti-Flag antibody up-shifted F-RXR homodimer.

Figure 2 shows the characterization of 9-cis-RA induced RXR homodimer on TREpal.

(a) Cooperative binding of 9-cis-RA induced RXR $\alpha$  homodimer. Formation of RXR-DNA complex at different receptor concentrations in the absence or presence of  $10^{-7}$  M 9-cis-RA was analyzed by gel retardation assay using labeled TREpal as probe. Open triangle indicates the nonspecific binding of an unprogrammed reticulocyte lysate. Arrows indicate the specific RXR binding complex.

(b) Quantitation of the RXR binding complex at different receptor concentrations in the presence of 9-cis-RA. Gel slices containing RXR binding complex in the presence of 9-cis-RA shown in Figure 2(a) were excised and counted in a scintillation counter and plotted.

(c) 9-cis-RA concentration-dependent binding of RXR homodimer on TREpal. Equal amounts of *in vitro* synthesized RXR protein were incubated with indicated concentrations of 9-cis-RA. The reaction mixtures were then analyzed by gel retardation assay using labeled TREpal as a probe. Open triangles indicate the nonspecific binding of unprogrammed reticulocyte lysate. Arrows indicate the specific RXR binding complex.

Figure 3 shows 9-cis-RA induces RXR homodimer binding on RXR-specific response elements.

(a) Nuclear receptor binding elements used in this study. These oligonucleotides were synthesized with appropriate restriction sites at both ends as indicated by the small letters. Sequences that are closely related to the AGG/TTCA motif are indicated by arrows.

5

(b) The effect of 9-cis-RA on RXR homodimer binding of ApoAI-RARE or CRBPII-RARE was analyzed essentially as described in Fig. 1a. Lane 1 represents the nonspecific binding of unprogrammed reticulocyte lysate, which are indicated by the open triangles. Solid 10 triangles indicate the RAR/RXR heterodimer complex. Arrows indicate 15 the specific RXR binding complex.

Figure 4 shows response element specific binding of RXR homodimer. The effect of 9-cis-RA on RXR binding on RA specific 15 response elements (a), T, specific response elements (b), or estrogen specific response element (c) was analyzed by gel retardation assays as described in Figure 1a. For comparison, the binding of RXR/RAR heterodimer (a), RXR/TR heterodimer (b) or estrogen receptor (c) is shown. Open triangles indicate the nonspecific binding of 20 unprogrammed reticulocyte lysate. Solid triangle indicates the RAR/RXR heterodimer complex (a), TR/RXR heterodimer complex (b) or ER complexes (c).

Figure 5 shows RXR homodimerization occurs in solution. 25 <sup>35</sup>S-labeled in vitro synthesized RXR $\alpha$  proteins were incubated with partially purified bacterially expressed Flag-RXR (F-RXR) (+) or similarly prepared glutathione transferase control protein (-) either in the presence or absence of response elements or chemical cross-linker DSP as indicated. After incubation, either anti-Flag antibody 30 (F) or nonspecific preimmune serum (NI) was added. 10<sup>-7</sup> M 9-cis-RA was maintained during working process. The immune complexes were washed in the presence of 10<sup>-7</sup> M cis-RA, boiled in SDS sample buffer and separated on a 10% SDS-PAGE. The <sup>35</sup>S-labeled in vitro synthesized RXR $\alpha$  protein is shown in the right panel.

35

Figure 6 shows transcriptional activation of RXR and RAR $\alpha$ : RXR heterodimers by 9-cis-RA on natural response elements. CV-1 cells

were cotransfected with 100 ng of the reporter plasmids (a) TREpal-tk-CAT (b)  $\beta$ RARE-tk-CAT (c) ApoAI-RARE-tk-CAT and (d) CRBPII-RARE-tk-CAT and 5 ng of empty pECE expression vector, pECE-RXR $\alpha$ , pECE RAR $\alpha$  or combination of both as indicated. Transfected cells were treated with

5 no hormone (open bars),  $10^{-7}$  M RA (shadowed bars) or  $10^{-7}$  M 9-cis-RA (dark shadowed bars). The results of a representative experiment performed in duplicate are shown.

Figure 7 shows RXR $\alpha$ -dependent transactivation of reporter  
10 constructs (a) TREpal-tk-CAT (10) or (b) CRBPII-tk-CAT (10) by  
9-cis-RA or retinoids SR11203, SR11217, SR11234, SR11235, SR11236, and  
SR11237. Results of a representative experiment carried out four  
times are shown. In four independent experiments, induction profiles  
did not vary significantly. CAT activity was normalized for  
15 transfection and harvesting efficiency by measuring the enzymatic  
activity derived from the cotransfected  $\beta$ -galactosidase expression  
plasmid (pCH110, Pharmacia).

## DETAILED DESCRIPTION OF THE INVENTION

## Definitions and Nomenclature:

Before the present compounds, compositions and methods are disclosed and described, it is to be understood that this 5 invention is not limited to specific synthetic methods, specific pharmaceutical carriers, or to particular pharmaceutical formulations or administration regimens, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be 10 limiting.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates 15 otherwise. Thus, for example, reference to "a bicyclic aromatic compound" includes mixtures of bicyclic aromatic compounds, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

20 In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

The term "alkyl" as used herein refers to a branched 25 or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like. Preferred alkyl groups herein contain 1 to 12 carbon atoms. The term "lower alkyl" intends an alkyl group of one to six carbon 30 atoms, preferably one to four carbon atoms. The term "cycloalkyl" intends a cyclic alkyl group of three to eight, preferably five or six, carbon atoms.

The term "alkoxy" as used herein intends an alkyl 35 group bound through a single, terminal ether linkage; that is, an "alkoxy" group may be defined as -OR where R is alkyl as defined

above. A "lower alkoxy" group intends an alkoxy group containing one to six, more preferably one to four, carbon atoms.

The term "alkylene" as used herein refers to a di-  
5 functional saturated branched or unbranched hydrocarbon chain  
containing from 1 to 24 carbon atoms, and includes, for example,  
methylene (-CH<sub>2</sub>-), ethylene (-CH<sub>2</sub>-CH<sub>2</sub>-), propylene (-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-), 2-  
methylpropylene [-CH<sub>2</sub>-CH(CH<sub>3</sub>)-CH<sub>2</sub>-], hexylene [-(CH<sub>2</sub>)<sub>6</sub>-] and the like.  
"Lower alkylene" refers to an alkylene group of 1 to 6, more  
10 preferably 1 to 4, carbon atoms. The term "cycloalkylene" as used  
herein refers to a cyclic alkylene group, typically a 5- or 6-membered  
ring.

The term "alkene" as used herein intends a mono-  
15 unsaturated or di-unsaturated hydrocarbon group of 2 to 24 carbon  
atoms. Preferred groups within this class contain 2 to 12 carbon  
atoms. Asymmetric structures such as (AB)C=C(CD) are intended to  
include both the E and Z isomers. This may be presumed in structural  
formulae herein wherein an asymmetric alkene is present, or it may be  
20 explicitly indicated by the bond symbol ~~~~~.

"Optional" or "optionally" means that the  
subsequently described event or circumstance may or may not occur, and  
that the description includes instances where said event or cir-  
25 cumstance occurs and instances where it does not. For example, the  
phrase "optionally substituted phenyl" means that the phenyl may or  
may not be substituted and that the description includes both  
unsubstituted phenyl and phenyl where there is substitution.

30 By the term "effective amount" of a compound as  
provided herein is meant a nontoxic but sufficient amount of the  
compound to provide the desired regulation of gene expression. As  
will be pointed out below, the exact amount required will vary from  
subject to subject, depending on the species, age, and general  
35 condition of the subject, the severity of the disease that is being  
treated, the particular bicyclic compound used, its mode of  
administration, and the like. Thus, it is not possible to specify an

exact "effective amount." However, an appropriate effective amount may be determined by one of ordinary skill in the art using only routine experimentation.

5 By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected bicyclic compound without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components 10 of the pharmaceutical composition in which it is contained.

"Eliciting," "modulating" or "regulating" selective gene expression is intended to mean that a compound is capable of acting as an activator or an antagonist of gene expression by a particular 15 receptor, i.e., a receptor in the retinoic acid family.

The "retinoic acid family" of receptors, also termed "retinoid receptors," is intended to encompass retinoic acid receptors, retinoid X receptors, vitamin D receptors and thyroid 20 hormone receptors. The aforementioned three groups of receptors may also be loosely referred to herein as "retinoic acid receptors," i.e., such that the term includes retinoid X receptors, vitamin D receptors and thyroid hormone receptors in addition to retinoic acid receptors themselves.

25 "Bridged, bicyclic aromatic compounds" intends all compounds encompassed by the structure of formula (I). These compounds may also be termed "retinoids," with that term further intended to include such species as retinoic acid and 9-cis retinoic 30 acid.

The invention provides a method of screening a substance for the ability to affect the formation of an RXR homodimer comprising combining the substance and a solution containing RXRs and determining 35 the presence of a homodimer formation. The presence of homodimer formation can, for example, be determined by detecting the activation of transcription by the RXR homodimer or by coprecipitation. The

affect can be the induction of homodimer formation, for example, an activity similar to that activated by 9-cis-RA or an activity which selectively activates homodimer formation over heterodimer formation. By "selectively activates" is meant a compound which activates 5 homodimer formation but does not significantly activate heterodimers. Alternatively, "selectively activates" includes compounds which activate heterodimer formation but do not significantly activate homodimer formation. "Significant activation" includes activation that is sufficient to cause a harmful pharmacological effect on the 10 subject. The affect can also be the inhibition of homodimer formation. Examples of inhibition include a substance which competes for 9-cis-RA binding to the receptor but itself does not activate or induce dimerization or which binds 9-cis-RA to block its activity. Such screening of substances is routinely carried out given the 15 subject discovery of homodimer formation. In particular assays set forth below can generally be used for screening by merely substituting the substance of interest for 9-cis-RA. A good starting point for screening such "substances" is the activity of 9-cis-RA described herein. The substituents on 9-cis-RA can be varied to make 9-cis-RA 20 analogs and screened in the method to determine any increase or decrease in homodimer formation. However, any substance can be screened in this assay to determine any affect on homodimer formation. Such compounds can then be used to promote homodimer formation and gene transcription in a cell. A cell as used herein includes cells 25 found either in vitro or in vivo. Thus, the compounds can be administered to a human subject to effect RXR homodimer formation and promote transcription of a gene activated by an RXR homodimer. Such compounds are set forth in the Examples.

30 The data set forth herein utilizes RXR $\alpha$ . However, given the high homology between RXR $\alpha$ ,  $\beta$  and  $\gamma$ , each protein should form homodimers and have the activity described for RXR $\alpha$  homodimers. Relatedly, homodimers can form between different RXRs. For example, 35 homodimers can form between RXR $\alpha$  and RXR $\beta$  or between RXR $\beta$  and RXR $\gamma$  or between RXR $\alpha$  and RXR $\gamma$ . The activity of these homodimers can be confirmed using the methods set forth herein.

The invention also provides a method of screening a substance for an effect on an RXR homodimer's ability to bind DNA comprising combining the substance with the homodimer and determining the effect of the compound on the homodimer's ability to bind DNA.

5 For example, compounds which might bind the homodimer or bind the DNA response element recognized by an RXR homodimer can be screened in this method.

The invention further provides a method of inhibiting an 10 activity of an RXR-containing heterodimer comprising increasing the formation of an RXR homodimer, thereby preventing the RXR from forming a heterodimer and preventing the resulting heterodimer activity. The activity can be any activity but is generally the activation or repression of transcription. The activity can be blocked, for 15 example, by utilizing RXRs to form homodimers which otherwise would be available to form heterodimers. Since the number of heterodimers are decreased, the activity of the heterodimers is decreased. In one example, the RXR heterodimer is comprised of thyroid hormone receptor and RXR. The activity of the RXR/TR heterodimer was decreased. Other 20 heterodimers can be tested using standard methods given the teaching set forth herein.

The invention also provides a method of inhibiting an activity of an RXR receptor homodimer comprising preventing the 25 formation of the RXR homodimer. Such inhibition can be obtained, for example, by inhibiting 9-cis-RA or the transcription or activation by 9-cis-RA. The activity inhibited is generally the activation or repression of transcription.

30 The invention also provides a method of inhibiting an activity of an RXR homodimer comprising preventing the binding of the RXR homodimer to its response element. For example, the activity of a receptor which competes for the same response element can be promoted. In general, the activity which is inhibited is the activation or 35 repression of transcription.

The invention still further provides a method of determining an increased probability of a pathology associated with RXR homodimer formation comprising detecting a modulation of RXR homodimer formation in the subject when compared to a normal subject.

5 The modulation can be an increase or a decrease in homodimer formation. Such a modulation can result, for example, from a mutated RXR. The decrease can be detected by an assay for the homodimers in a sample or by detecting mutations known to decrease homodimer formation.

10

Relatedly, the invention provides a method of treating a pathology associated with RXR homodimer formation in a subject comprising modulating homodimer formation in the subject. The modulation can be an increase or a decrease depending on the 15 pathology. Such an increase can be accomplished, for example, utilizing compounds which promote RXR transcription. The pathology can be associated with the skin, e.g., acne and psoriasis. In addition, the pathology can be a cancer. The exact amount of such compounds required will vary from subject to subject, depending on the 20 species, age, and general condition of the subject, the severity of the disease that is being treated, the particular compound used, its mode of administration, and the like. Thus, it is not possible to specify an exact activity promoting amount. However, an appropriate amount may be determined by one of ordinary skill in the art using 25 only routine experimentation given the teachings herein.

The invention also provides a purified RXR homodimer. By "purified" is meant free of at least some of the cellular components associated with RXR homodimers in a natural environment.

30

The invention further provides a method of screening a response element for binding with a RXR homodimer comprising combining the response element with the RXR homodimer and detecting the presence of binding. The presence of binding can be determined by a number of 35 standard methods. In one method, binding is detected by the transcriptional activation of a marker which is operably linked to the

response element. By "operably linked" is meant the marker can be transcribed in the presence of the transcriptional activator.

The invention grows out of our study of the effects of the 5 natural vitamin A derivative 9-cis-RA on retinoid receptor DNA binding and transcriptional activation. In contrast to all-trans-RA, the 9-cis analog dramatically enhances RXR $\alpha$  binding at 10<sup>-9</sup> to 10<sup>-8</sup>M concentrations to several RXR-specific RAREs but not to natural TREs or the ERE. The effect is specific to RXR since 9-cis-RA did not 10 induce binding of RAR $\alpha$ ,  $\beta$  or  $\gamma$  to response elements (Fig. 1, Fig. 3). Judging from the migration pattern in the gel shift assays, we assume that 9-cis-RA induces homodimer formation, although a larger complex containing an RXR trimer or tetramer can occur (particularly in the case of the CRBPII-RARE). Such trimer or tetramer formation can be 15 tested using the methods set forth herein.

RXR $\alpha$  homodimers exert response element specificity distinct from heterodimers. The rCRBPI response element did not interact with RXR homodimers, while the CRBPII response element, the 20 only natural RARE identified so far that contains perfect repeats, was a strong binder of 9-cis-RA induced RXR $\alpha$  homodimers. It has been shown previously that this response element is well activated by RXR $\alpha$ <sup>24,30</sup>. Although this response element is also bound effectively by the RXR $\alpha$ -RAR $\alpha$  heterodimer (Fig. 3)<sup>27,29</sup>, the heterodimer appears to have 25 a repressor function<sup>30</sup>.

The results obtained with the transcriptional activation studies agree well with the DNA binding studies although CV-1 cells, like all other mammalian tissue culture cells tested, contain 30 endogenous retinoid receptors that can partially obscure effects. Nonetheless, response elements that strongly bind 9-cis-RA-RXR $\alpha$  homodimers also responded strongly to cotransfected RXR $\alpha$  in the presence of 9-cis-RA, whereas response elements that did not bind well to RXR $\alpha$  homodimers, like the rCRBPI-RARE or the MHC-TRE, could not be 35 activated by RXR $\alpha$  alone.

It is generally believed that the dimerization - homodimerization or heterodimerization - of nuclear hormone receptors is critical for high affinity interaction of the receptors with their cognate response elements. RXRs exist mainly as monomer in solution<sup>16</sup> and require high concentrations or the presence of RARs, TRs or VDR to display effective DNA binding activity<sup>13,15,16,26-30</sup>. The observation of the enhanced cooperative RXR DNA binding activity in the presence of 9-cis-RA demonstrates that 9-cis-RA induced the formation of RXR homodimers which have an increased affinity for DNA. Thus, binding of 9-cis-RA to RXR can induce a conformational change, which allows homodimerization to occur. It is interesting that although 9-cis-RA and RA can bind to RAR,<sup>24,25</sup> they do not induce RAR homodimer formation.

RXR $\alpha$  homodimer formation can occur in solution in the absence of DNA. Thus, when 9-cis-RA becomes available to cells, the equilibrium between monomeric and dimeric receptors is changed and an additional species, the RXR homodimer can be formed, allowing for novel response pathways. The concept of ligand-induced homodimer binding as observed by *in vitro* gel shift assay has not been previously observed for nuclear receptors with the exception of a mutated estrogen receptor (ER-val-400)<sup>44,45</sup>.

For the related TRs, a ligand effect has been reported on homodimer binding,<sup>46,47</sup> however the ligand (T3) reduced homodimer response element interaction. Since the carboxy terminal half of TRs and RARs encodes both ligand as well as dimerization functions<sup>36,46,48</sup>, the strong effect of the ligand on dimerization as observed here is not completely surprising. However, the specificity of the effect is quite dramatic since only homodimer but not heterodimer formation appears to be affected. An overall picture emerges where the carboxy terminal region of a receptor through its intermixed domains (that also includes a transcriptional activation region)<sup>49</sup> allows for multiple activities of individual receptors that may also include interactions with other regulatory proteins<sup>50</sup>.

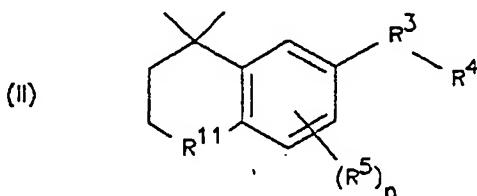
35

The data presented in this application clearly demonstrate the central role of the RXRs, having dual functions that allow them to

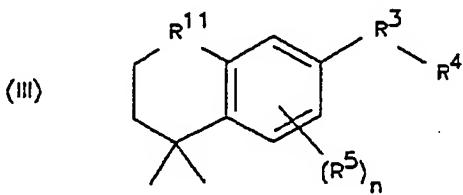
act as auxiliary receptors for three classes of hormone receptors, the RARs, TRs and VDRs through heterodimerization. The two functions of RXR - homodimerization and heterodimerization - represent two distinct transcriptional regulatory controls that can be expected to affect 5 distinct physiological processes. Thus, 9-cis-RA can have therapeutic properties distinct from that of all-trans-RA.

The novel compounds provided herein are those defined by structural formula (I) above. Preferred compounds within 10 this generic structure include

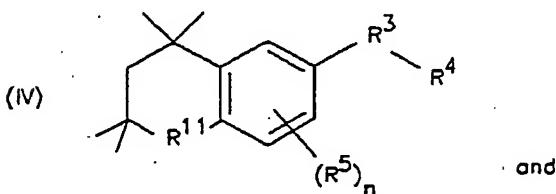
15



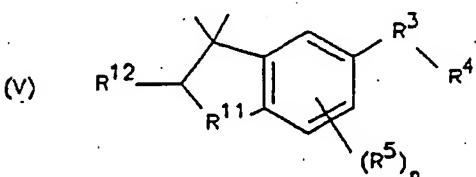
20



25



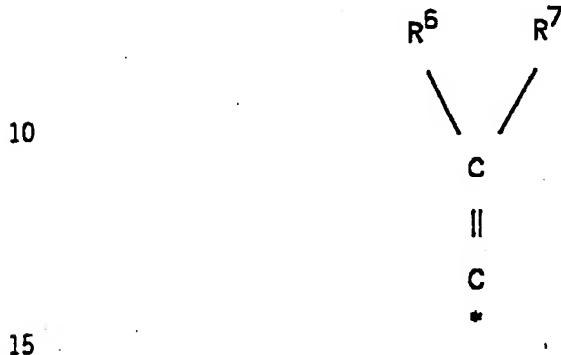
30



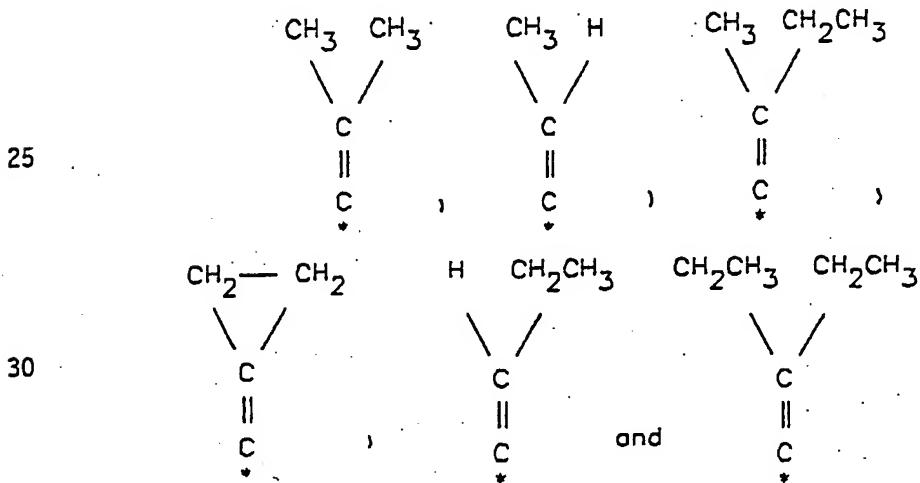
35

where  $R^{11}$  is selected from the group consisting of O, S,  $(CH_3)_2C$  and  $CH_2$ , and  $R^{12}$  is hydrogen or methyl. Particularly preferred compounds within this group are as shown in structural formula (II).

5 Preferred  $R^3$  substituents, when  $R^3$  has the general  
structure

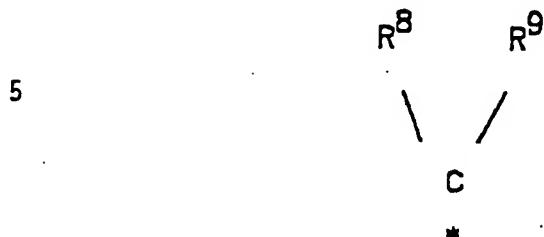


are selected from the group consisting of  
20



20

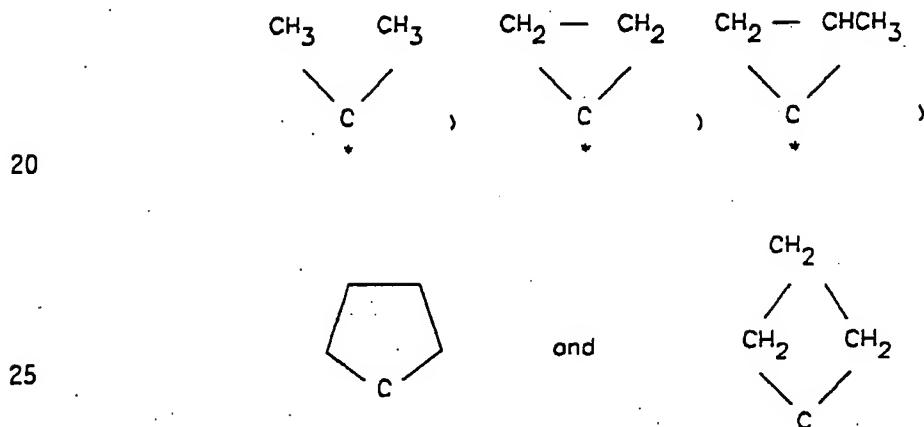
Preferred R<sup>3</sup> substituents, when R<sup>3</sup> has the general  
structure



10

are selected from the group consisting of

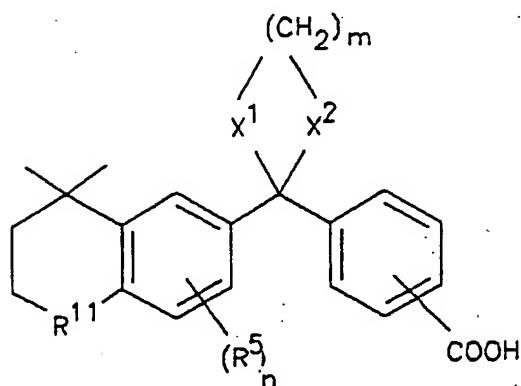
15



Examples of preferred structures encompassed by formula  
(II) thus include

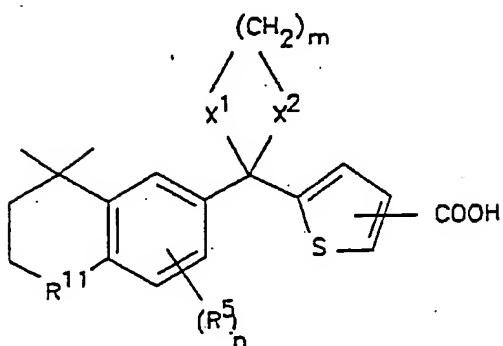
5

10



15

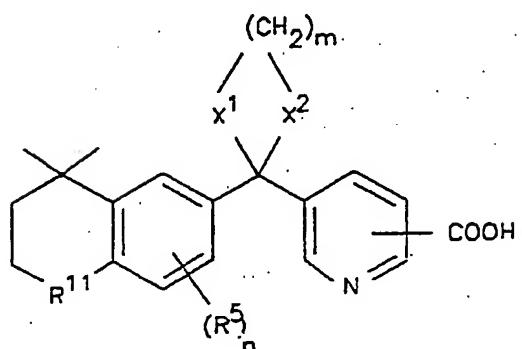
20

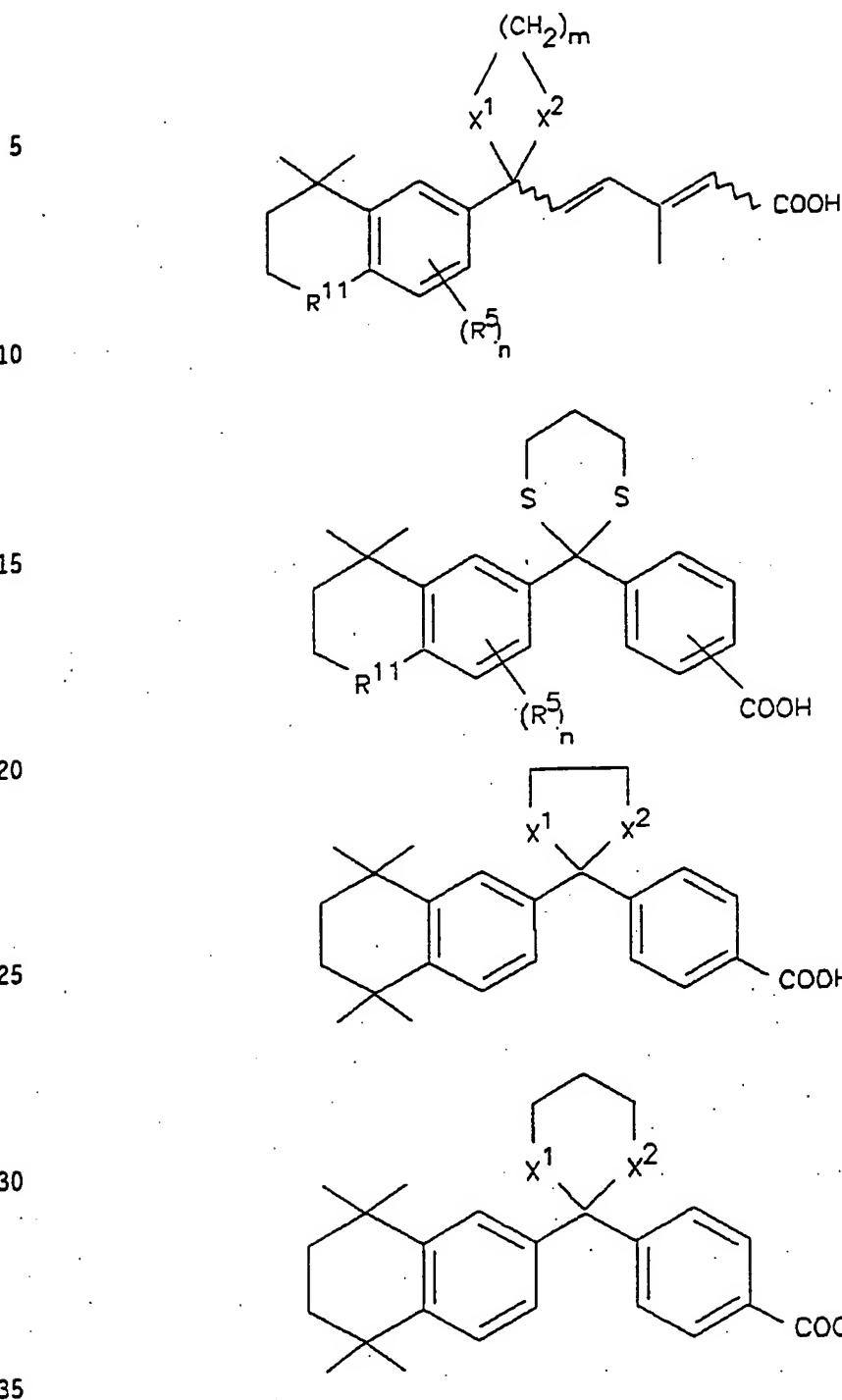


25

30

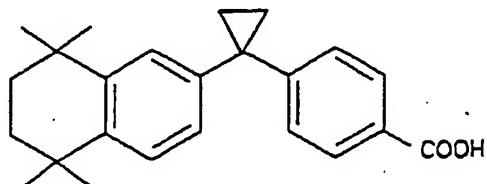
35



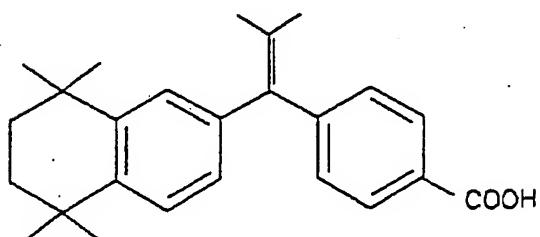


Examples of specific and particularly preferred compounds within the class of compounds defined by formula (II) include:

5

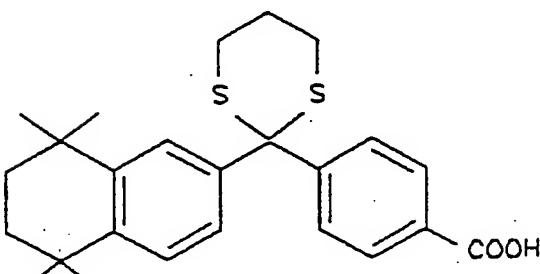


10



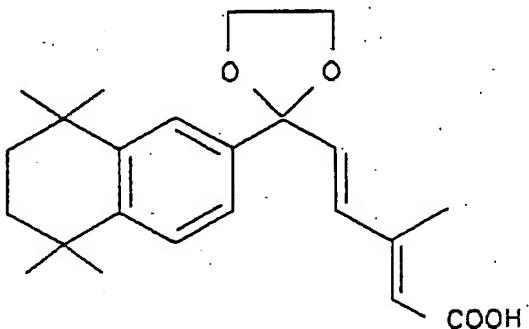
15

20



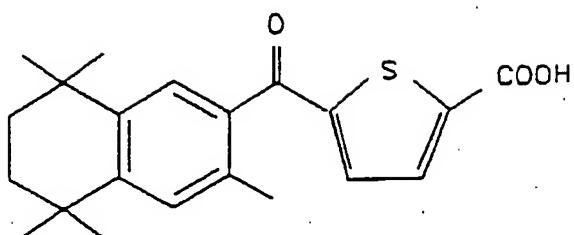
25

30



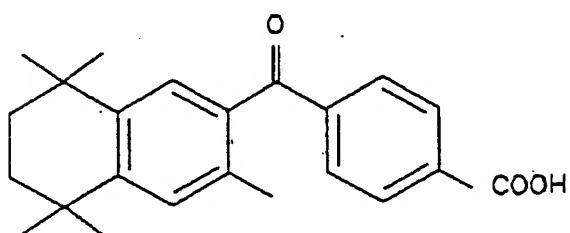
35

5



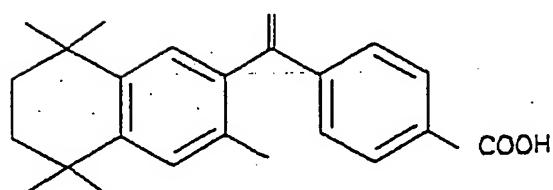
10

15



20

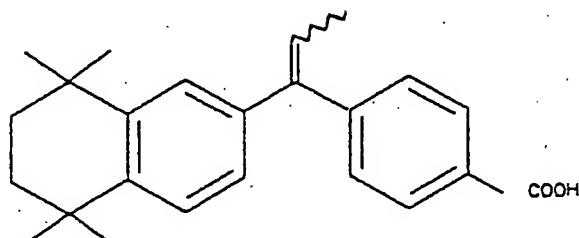
25



and

30

35



The invention also encompasses pharmaceutically acceptable nontoxic ester, amide and salt derivatives of those compounds of formula (I) containing a carboxylic acid moiety.

5       Pharmaceutically acceptable salts are prepared by treating the free acid with an appropriate amount of pharmaceutically acceptable base. Representative pharmaceutically acceptable bases are ammonium hydroxide, sodium hydroxide, potassium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, ferrous hydroxide, 10 zinc hydroxide, copper hydroxide, aluminum hydroxide, ferric hydroxide, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, lysine, arginine, histidine, and the like. The reaction is conducted in water, alone or in combination with an inert, 15 water-miscible organic solvent, at a temperature of from about 0°C to about 100°C, preferably at room temperature. The molar ratio of compounds of structural formula (I) to base used are chosen to provide the ratio desired for any particular salts. For preparing, for example, the ammonium salts of the free acid starting material--a 20 particular preferred embodiment herein--the starting material can be treated with approximately one equivalent of pharmaceutically acceptable base to yield a neutral salt. When calcium salts are prepared, approximately one-half a molar equivalent of base is used to yield a neutral salt, while for aluminum salts, approximately one- 25 third a molar equivalent of base will be used.

Ester derivatives are typically prepared as precursors to the acid form of the compounds--as illustrated in the Examples below-- 30 and accordingly may serve as prodrugs. Generally, these derivatives will be lower alkyl esters such as acetate, propionate, and the like. Amide derivatives -(CO)NH<sub>2</sub>, -(CO)NHR and -(CO)NR<sub>2</sub>, where R is lower alkyl, may be prepared by reaction of the carboxylic acid-containing compound with ammonia or a substituted amine (as illustrated in 35 Example VII below).

**Synthetic Methods:**

The compounds of the invention may be readily synthesized using techniques generally known to synthetic organic chemists. Suitable experimental methods for making and derivatizing bridged 5 bicyclic aromatic compounds are described, for example, in the Maignan et al. patents summarized above, the disclosures of which patents are hereby incorporated by reference. Methods for making specific and preferred compounds of the present invention are described in detail in Examples III - XVII below.

10

**Utility and Administration:**

The compounds of the invention defined by structural formula (I), including the pharmacologically acceptable esters, amides or salts thereof, are useful to elicit and/or regulate selective gene 15 expression by receptors in the retinoic acid family and to control cell differentiation processes regulated by retinoids, vitamin D and/or thyroid hormone. As noted above, the compounds of the invention are thus useful for treating acne, leukemia, psoriasis, skin aging, bone calcification and energy levels, as well as other 20 indications related to cellular processes regulated by retinoic acid, vitamin D, thyroid hormone and 9-cis-retinoic acid.

The compounds of the invention may be conveniently formulated into pharmaceutical compositions composed of one or more of the 25 compounds in association with a pharmaceutically acceptable carrier. See, e.g., Remington's Pharmaceutical Sciences, latest edition, by E.W. Martin (Mack Publ. Co., Easton PA) discloses typical carriers and conventional methods of preparing pharmaceutical compositions that may be used in conjunction with the preparation of formulations of the 30 inventive compounds.

The compounds may be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, topically, transdermally, or the like, although oral or 35 topical administration is typically preferred. The amount of active compound administered will, of course, be dependent on the subject being treated, the subject's weight, the manner of administration and

the judgment of the prescribing physician. Generally, however, dosage will approximate that which is typical for the administration of retinoic acid, and will preferably be in the range of about 2  $\mu\text{g}/\text{kg}/\text{day}$  to 2  $\text{mg}/\text{kg}/\text{day}$ .

5

Depending on the intended mode of administration, the pharmaceutical compositions may be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, suspensions, lotions, creams, gels, 10 or the like, preferably in unit dosage form suitable for single administration of a precise dosage. The compositions will include, as noted above, an effective amount of the selected drug in combination with a pharmaceutically acceptable carrier and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, 15 adjuvants, diluents, etc.

For solid compositions, conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, 20 cellulose, glucose, sucrose, magnesium carbonate, and the like. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc., an active compound as described herein and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, 25 ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, 30 triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see Remington's Pharmaceutical Sciences, referenced above.

35

For oral administration, fine powders or granules may contain diluting, dispersing, and/or surface active agents, and may be presented in water or in a syrup, in capsules or sachets in the dry

state, or in a nonaqueous solution or suspension wherein suspending agents may be included, in tablets wherein binders and lubricants may be included, or in a suspension in water or a syrup. Where desirable or necessary, flavoring, preserving, suspending, thickening, or 5 emulsifying agents may be included. Tablets and granules are preferred oral administration forms, and these may be coated.

Parenteral administration, if used, is generally characterized by injection. Injectables can be prepared in conventional 10 forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release 15 system, such that a constant level of dosage is maintained. See, e.g., U.S. Patent No. 3,710,795, which is incorporated by reference herein.

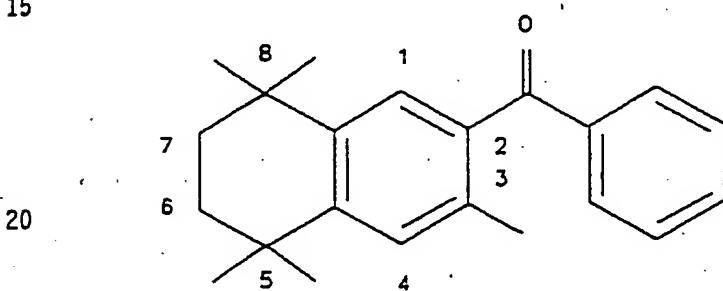
---

## Experimental

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and 5 description of how the methods, compositions, and compounds claimed herein are made and evaluated, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted 10 for. Unless indicated otherwise, parts are parts by weight, temperature is in °C and pressure is at or near atmospheric.

In describing the location of groups and substituents, the following numbering system will be employed throughout the examples:

15



20

25

EXAMPLE I

## Identification of homodimer formation

30

9-cis-RA induces RXR homodimer binding on the TREpal

Although RXRs have been shown to bind RA response elements (RAREs) when used at high concentrations,<sup>28,30,31</sup> more recent 35 investigations revealed that RXR exists mainly as monomers in solution<sup>10</sup> and that effective DNA interaction requires heterodimer formation with RARs or TRs or VDR<sup>15,16,26-29</sup>. Binding of the heterodimers

to a variety of response elements was found to be ligand independent<sup>32</sup>. The newly discovered natural RA isomer, 9-cis-RA, has been reported to be an effective activator of RXRs in *Drosophila Schneider* cells that are known to contain neither RAR nor TRs<sup>17,24</sup>. If 9-cis-RA is indeed a true ligand for RXRs, one might expect that this ligand modulates RXR response element interaction. We therefore investigated the effect of 9-cis-RA on RXR $\alpha$  binding to the palindromic TRE (TREpal), an RXR responsive element<sup>14</sup>, in the absence and presence of coreceptors (TRs and RARs).

10

The cloning of the receptor cDNAs for RXR $\alpha$ , RAR $\beta$ , or TR $\alpha$  into the pBluescript (Stratagene, San Diego, CA) have been described previously<sup>26</sup>. Flag-RXR $\alpha$  was constructed as described previously<sup>33</sup> by ligation of a double-stranded oligonucleotide containing an ATG codon and a DNA sequence encoding Flag (Arg-Tyr-Lys-Asp-Asp-Asp-Lys) [SEQ ID NO:1] to the N-terminus of RXR $\alpha$ . The fusion product was then cloned into pBluescript. The synthesis of receptor proteins using *in vitro* transcription/translation system and gel retardation assays using synthesized receptor proteins and the double-stranded TREpal<sup>34</sup> were as described<sup>33</sup>. 9-cis-RA (m.p. 184-187°C) was prepared from 9-cis-retinal by a two-step sequence of MnO<sub>2</sub> oxidation in the presence of HOAc-MeOH<sup>35</sup> to give the methyl ester (69%) followed by hydrolysis (80%) in 0.5 N KOH in 25% aq. MeOH and crystallization (MeOH); HPLC (Novapak C<sub>18</sub>, 32% MeCN, 27% MeCH, 16% I-PrOH, 24% H<sub>2</sub>O, 1% HOAc, 1.9 mL/min, 260nm) *t*<sub>r</sub> 15.4 min (100%). To analyze the effect of hormones, the receptor proteins were incubated with appropriate concentrations of hormone at room temperature for 30 min before performing the DNA binding assay. When anti-Flag antibody (Immunex, Seattle, WA) was used, 1  $\mu$ l of the antiserum was incubated with receptor protein for another 30 min at room temperature before performing the DNA binding assay.

While in the absence of ligand, RXR alone did not bind effectively to the TREpal and required TR or RAR for response element interaction, binding of RXR was dramatically increased in the presence of 9-cis-RA (Fig. 1a) and did not require TRs or RARs. The RXR-specific band observed in the presence of 9-cis-RA was as prominent as

the bands obtained with the heterodimeric TR-RXR and RAR-RXR complexes. The RXR complex migrated more slowly than the TR-RXR complex at a position very similar to that of the RAR-RXR heterodimer-TREpal complex. These data therefore demonstrate that 9-cis-RA induces RXR homodimer formation. Due to migration of the RAR-RXR complex at the same position as the 9-cis-RA induced RXR homodimer complex, we were unable to determine in this experiment whether both complexes were formed. Remarkably, all-trans-RA when added at a concentration of 10<sup>-6</sup> M, also induced to some degree RXR $\alpha$  homodimer binding whereas T<sub>3</sub> did not. Although the RA effect could be observed with several freshly prepared RA solutions, it is not clear at this point whether this homodimer formation in the presence of RA is due to 9-cis-RA impurities in the all-trans-RA solutions used here, or is a direct effect of all-trans-RA (see below). Interestingly, although it was reported that 9-cis-RA is capable of binding to RAR<sup>24</sup>, unlike its effect on RXR, 9-cis-RA was not found to induce RAR homodimer binding to the TREpal.

To provide further evidence that the observed complex in the presence of 9-cis-RA indeed was an RXR complex, we performed the DNA binding experiment with Flag-RXR, a derivative that carries an 8 amino acid aminoterminal epitope recognized specifically by anti-Flag ( $\alpha$ F) antibody<sup>33</sup>. As shown in Fig. 1b,  $\alpha$ F supershifted the 9-cis-RA induced complexes, while a nonspecific antibody did not. This proves that the complex observed with the TREpal in the presence of 9-cis-RA indeed contained RXR protein. To examine how dependent the 9-cis-RA induced homodimer formation is on the concentration of RXR protein, increasing concentrations of *in vitro* translated RXR protein were mixed with the labeled TREpal in the presence or absence of 9-cis-RA (Fig. 2a). When these mixtures were analyzed by the gel retardation assay, a strong cooperative effect in homodimeric DNA binding was seen, positively dependent on the RXR $\alpha$  protein concentration (Fig. 2a,b). Although slight binding of RXR can be observed when high concentrations of RXR were used, 9-cis-RA was required for efficient complex formation at all receptor concentrations used. We further determined the concentrations of 9-cis-RA required for homodimer complex formation at all receptor concentrations used. We further

determined the concentrations of 9-cis-RA required for homodimer complex formation and observed a significant effect already at 10<sup>-9</sup> M while optimal binding was seen at 10<sup>-8</sup> M (Fig. 2c). Thus, effective RXR homodimer DNA interaction is dependent on RXR protein 5 concentration and can occur at low levels of 9-cis-RA.

9-cis-RA induced homodimeric interaction with specific response elements

10 RXR containing heterodimers have a highly specific interaction with various natural response elements in that TR-RXR heterodimers only bind strongly to TREs but not to RAREs, whereas the opposite is true for RAR-RXR heterodimers<sup>15,32</sup>. We examined the sequence requirement of DNA binding of 9-cis-RA induced RXR homodimer 15 using a number of natural and synthetic response elements (Fig. 3a).

Gel retardation assays using in vitro synthesized receptor protein are described in the Fig. 1 legend. The following oligonucleotides and their complements were used as probes in Figs. 3 20 and 4. ApoAI-RARE, a direct repeat response element with 2 bp spacer<sup>31</sup>, gatcAGGGCAGGGTCAAGGGTTCAGTgatc [SEQ ID NO:2]; CRBPII-RARE, a direct repeat RXR-specific response element with 1 bp spacer<sup>30</sup>, gatcCAGGTACAGGTACAGGTACAGTTCAAgatc [SEQ ID NO:3]; BRARE, a direct repeat of RA response element present in RARB promoter<sup>36,37</sup>, 25 gatctGTAGGGTTACCGAAAGTTCACTCAGatc [SEQ ID NO:4]; CRBPI-RARE, a direct repeat RA specific response element present in rat CRBPI promoter<sup>38</sup>, gatccAGGTCAAAAGTCAGgatc [SEQ ID NO:5]; MHC-TRE, a direct repeat T, specific response element present in rat  $\alpha$ -myosin heavy chain gene<sup>40</sup>, gatcCTGGAGGTGACAGGAGGACAGCgatc [SEQ ID NO:6]; ME-TRE, a direct repeat 30 T, specific response element present in the rat malic enzyme gene<sup>41</sup>, gatcCAGGACGTTGGGGTTAGGGGAGGACAGTGGgatc [SEQ ID NO:7]; DR-4, an idealized direct repeat T, specific response element with 4 bp spacer<sup>39</sup>, gatcTCAGGTACCTCAGGTCAgatc [SEQ ID NO:8]; DR-5, an idealized direct repeat RA specific response element with 5 bp spacer<sup>39</sup>, 35 gatcTCAGGTACTCAGGTCAgatc [SEQ ID NO:9]; ERE, a perfect palindromic ER response element<sup>42</sup>, gatcTCAGGTCACTGTGACCTGAgatc [SEQ ID NO:10]. The sequence of TREpal [SEQ ID NO:11] is shown for comparison.

ApoAI-RARE (a direct repeat response element that contains a 2 bp spacer), which has been suggested to be RXR-specific<sup>31</sup>, resulted in a strong RXR complex in the presence of 9-cis-RA and to a lesser degree with RA (10<sup>-6</sup> M). The RXR-RAR heterodimer also bound effectively to this response element. Since the heterodimer complex migrated at the same position as RXR homodimers, the effect of 9-cis-RA on RXR-RAR heterodimers cannot be clearly determined. RAR homodimer binding was not induced by 9-cis-RA (Fig. 3b). When we investigated 9-cis-RA induced RXR binding to another RXR responsive element<sup>30</sup>, the CRBPII-RARE, we observed a complex that migrated more slowly than the heterodimer (in the absence of ligand), while in the presence of 9-cis-RA and RAR, both homodimer and heterodimer binding appeared to be reduced in their intensity (Fig. 3); a similar effect was seen at high RA concentrations or when both 9-cis-RA and RA were present. 9-cis-RA also induced RXR interaction with the BRARE (Fig. 4a), the RAR response element from the human RARB promoter<sup>36,37</sup> that contains a 5 bp spacer. However, the RXR homodimer band was considerably weaker than the RAR-RXR heterodimer band at the protein concentrations used. Interestingly, another natural RARE derived from the rat CRBPI promoter<sup>38</sup>, that like the ApoAI-RARE contains a 2 bp spacer, did not show any binding of RXR in the presence of 9-cis-RA (Fig. 4a), indicating that the actual sequence of the repeated core motif is critical for RXR homodimer binding. Similarly, the DR-5-RARE, a perfect repeat element derived from the B-RARE<sup>39</sup> did not exhibit interaction with RXR in the presence of 9-cis-RA, while it interacted strongly with RXR when RARE was present (Fig. 4a).

To examine whether RXR homodimer binding is specific to certain RAREs, we also performed gel shift experiments with the T<sub>3</sub> response elements from the rat  $\alpha$ -myosin heavy chain promoter (MHC-TRE)<sup>40</sup>, the rat malic enzyme (ME-TRE)<sup>41</sup> and the perfect repeat DR-4<sup>39</sup>. In all three cases, specific binding of RXR in the presence or absence of 9-cis-RA could not be observed, while all three response elements bound effectively TR/RXR heterodimers (Fig. 4b), consistent with the notion that these elements are not induced by retinoids. Similarly, the perfect palindromic ERE<sup>42</sup> also did not interact with RXR homodimers (Fig. 4c).

9-cis-RA induced homodimer formation occurs in the absence of DNA

It was reported the RXR exists mainly as monomer in solution<sup>16</sup>. An important question is whether 9-cis-RA induced RXR homodimers, like the RXR containing heterodimers, can form in solution in the absence of DNA. To address this question we took advantage of the Flag-RXR derivative that can be specifically precipitated with anti-Flag antibody while RXR wild type cannot.

Flag-RXR $\alpha$  was cloned in frame in the expression vector pGex 2T (Pharmacia) and was expressed in bacteria using the procedure provided by the manufacturer. Protein was partially purified on a prepacked glutathione sepharose 4B column (Pharmacia) and tested for its function by gel retardation assays and western blotting using anti-Flag antibody. Immunoprecipitation assay was performed essentially as described<sup>26</sup>. Briefly, 10  $\mu$ l of  $^{35}$ S-labeled in vitro synthesized RXR $\alpha$  protein was incubated with 5  $\mu$ l (approximately 0.1  $\mu$ g) of partially purified bacterially expressed Flag-RXR $\alpha$  fusion protein or similarly prepared glutathione transferase control protein in 100  $\mu$ l buffer containing 10<sup>-7</sup> M 9-cis-RA, 50 mM KCl and 10% glycerol for 30 min at room temperature. When assayed in the presence of chemical cross-linker or oligonucleotides, we added 2  $\mu$ l of 100 mM dithiobis succinimidylpropionate (DSP) dissolved in DMSO or 10 ng of oligonucleotide and continued the incubation at room temperature for 15 min. The reaction mixtures were then incubated with 1  $\mu$ l of anti-Flag antibody or nonspecific preimmune serum for 2 h on ice. Immune complexes were precipitated by adding 50  $\mu$ l of protein-A-sepharose slurry and mixing continuously in the cold room for 1 h. The immune complexes were washed extensively with cold NET-N buffer (20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM DTT, 0.5% NP-40) containing 10<sup>-7</sup> M 9-cis-RA, boiled in SDS sample buffer and resolved by SDS-polyacrylamide gel electrophoresis. The gel was fixed, dried and visualized by autoradiography.

When we mixed Flag-RXR with in vitro labeled  $^{35}$ S-RXR protein, the labeled RXR could be coprecipitated in the presence of anti-Flag antibody but not in the presence of nonspecific serum (Fig.

5). Coprecipitation efficiency was slightly increased in the presence of the ApoAI-RARE but not in the presence of the MHC-TRE. In addition, incubation with a crosslinker (DSP) further enhanced coprecipitation of the labeled RXR. In all cases, specific coprecipitation was only  
5 observed in the presence of 9-cis-RA. These data, therefore, give strong support to the assumption that 9-cis-RA induced RXR homodimer formation occurs in solution and does not require RXR-DNA interaction.

10 Response element specific transcriptional activation by 9-cis-RA and RXR $\alpha$

To investigate whether 9-cis-RA/RXR $\alpha$  homodimer response element interaction can be correlated with transcriptional activation of such response elements by the 9-cis-RA/RXR complex, we carried out  
15 a series of transient transfection assays in CV-1 cells, where we cotransfected receptor expression vectors with CAT reporter constructs that carried various response elements upstream of the tk promoter. CV-1 cells were transiently transfected using a modified calcium phosphate precipitation procedure as described previously<sup>43</sup>. CAT  
20 activity was normalized for transfection efficiency by measuring the enzymatic activity derived from the cotransfected  $\beta$ -galactosidase expression plasmid (pCH110, Pharmacia). The transfected cells were grown in the absence or presence of 10<sup>-7</sup> M 9-cis-RA or all-trans-RA.

25 With the TREpal containing reporter, we observed strong activation by RXR $\alpha$  in the presence of 9-cis-RA and little activation when RA was added. Activation could be further enhanced by cotransfection of RAR $\alpha$ . In this case, however, RA also functioned as an effective activator although not as efficiently as 9-cis-RA.  
30 Overall, activation by 9-cis-RA in the presence of RAR $\alpha$  and RXR $\alpha$  was approximately twice as strong as seen with RXR $\alpha$  alone, consistent with the DNA binding data where binding was observed by both the heterodimer and the homodimer in the presence of 9-cis-RA when both receptors were present (Fig. 6a). When we examined the BRARE (Fig.  
35 6b), we observed that this response element was highly activated by endogenous CV-1 cell receptors consistent with previous observations<sup>32,42</sup>, such that further activation by low concentrations of cotransfected receptors could not be observed. Interestingly,

however, 9-cis-RA was a more potent activator at  $10^{-7}$  M than RA. These data thus indicate that CV-1 cells contain endogenous retinoid receptor activity that is particularly active on the BRARE and is responsive to 9-cis-RA.

5

The ApoAI element containing reporter was also very effectively activated by RXR $\alpha$  in the presence of 9-cis-RA (Fig. 6c) while RA did not induce above the level obtained in the absence of RXR $\alpha$ . Similar to the TREP<sub>1</sub>, maximal activation was seen when both 10 receptors RXR $\alpha$  and RAR $\alpha$  were cotransfected. Under these conditions RA also led to a strong activation. In contrast, the CRBPI element, where we did not observe DNA binding by RXR in the presence of 9-cis-RA, also was not activated by RXR and 9-cis-RA in the transient 15 transfection studies (Fig. 6d) while RAR $\alpha$  alone led to significant activation that was mostly 9-cis-RA dependent. The heterodimer RAR $\alpha$ RXR $\alpha$  allowed maximal activation in the presence of 9-cis-RA. Not unexpectedly, no induction by RXR $\alpha$  and 9-cis-RA was observed on the 20 MHC-TRE, the ME-TRE or the ERE. These *in vivo* analyses showed a very significant correlation to the results obtained with the *in vitro* DNA binding studies, in that strong activation by RXR $\alpha$  in the presence of 9-cis-RA is only observed on the response elements that strongly interact with the 9-cis-RA induced RXR $\alpha$  homodimer.

#### 9-cis Retinoic acid inhibits activation by TR/RXR heterodimer

25

We have observed that a CAT reporter gene that is activated by a thyroid hormone receptor/RXR heterodimer in the presence of thyroid hormone (T<sub>3</sub>) can be inhibited by adding 9-cis-RA. This type of inhibition is most easily measured by using a 30 transfection assay.

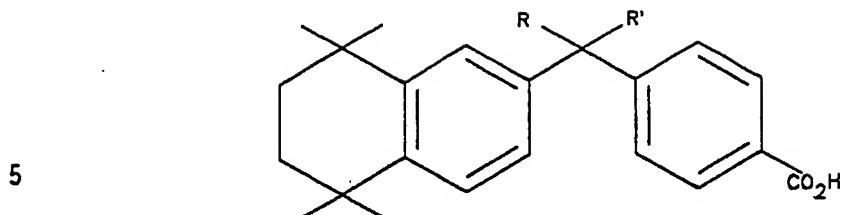
#### EXAMPLE II

##### Identification and selection of compounds which induce RXR activity

35

We used the TREP<sub>1</sub>-tk-reporter gene in a transient transfection assay essentially as described<sup>51</sup> to evaluate compounds for

induction of RXR activity. Briefly, CV-1 cells or Hep G2 cells were grown in DME medium supplemented with 10% fetal calf serum. Cells were plated at  $1.0 \times 10^5$  per well in a 24-well plate 16-24 h before transfection. In general, 100 ng of reporter plasmid, 150 ng of 5  $\beta$ -galactosidase expression vector (pCH110, Pharmacia), and variable amounts of receptor expression vector were mixed with carrier DNA (pBluescript) to 1,000 ng of total DNA per well. Chloramphenicol acetyl transferase (CAT) activity was normalized for transfection efficiency by the corresponding  $\beta$ -galactosidase activity as previously 10 described<sup>52</sup>. As shown in Example I, the TREP<sub>1</sub> represents a response element that is activated by both RAR/RXR heterodimers and RXR homodimers. When the RXR expression vector is cotransfected with the TREP<sub>1</sub>-tk-reporter gene into CV-1 cells, all-trans-RA does not 15 efficiently activate the reporter, whereas 9-cis-RA does. Evaluation of a series of retinoids indicated that several showed activity with RXR. The pharmacophoric elements of these structures were then combined and further modified to produce a subset of retinoids whose activation profiles for RXR were similar to that of 9-cis-RA. Induction curves for several retinoids active with RXR are shown in 20 Fig. 7a. Interestingly, while none of the active compounds revealed activity at  $10^{-8}$  M, all showed activities similar to 9-cis-RA at  $10^{-7}$  M. We next used a reporter gene carrying the RARE<sup>30</sup> of the cytoplasmic 25 retinol binding protein II (CRBPII), a response element that is only activated by RXR homodimers, but not by RAR/RXR heterodimers. The induction profiles obtained with this response element were similar to the TREP<sub>1</sub> responses (Fig. 7b). Thus, the synthetic retinoids SR11203, SR11217, SR11234, SR11235, SR11236, and SR11237 appeared to be effective activators of RXR $\alpha$ . The structures of the compounds are as follows:



|    |         |  |         |         |
|----|---------|--|---------|---------|
|    | SR11203 | R, R' = SCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> S | Ex. III | Cmp. 5  |
|    | SR11217 | R, R' = (CH <sub>3</sub> ) <sub>2</sub> C                  | Ex. IX  | Cmp. 20 |
| 10 | SR11234 | R, R' = SCH <sub>2</sub> CH <sub>2</sub> S                 | Ex. IV  | Cmp. 7  |
|    | SR11235 | R, R' = OCH <sub>2</sub> CH <sub>2</sub> S                 | Ex. VI  | Cmp. 12 |
|    | SR11236 | R, R' = OCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> O | Ex. VII | Cmp. 14 |
|    | SR11237 | R, R' = OCH <sub>2</sub> CH <sub>2</sub> O                 | Ex. V   | Cmp. 9  |

15 The activity rankings for this series of retinoids were the same for both the TREP<sub>1</sub> and CRBPII reporter genes. The ketal SR11237 was the most active, followed by the isopropylidene retinoid SR11217, the hemithioketal SR11235 and the thioketal SR11234. The dithiane SR11203 and dioxane SR11236 had the lowest activity.

20 Conformational analysis indicated that these retinoids had spatial orientations of the lipophilic head and carboxyl terminus that were similar to those of *9-cis*-RA and that activity could be related to the length and volume of the substituent group (CRR') linking the tetrahydronaphthalene and phenyl ring systems.

25 Given the showing in Example I that *9-cis*-RA specifically activates RXR $\alpha$  by inducing RXR $\alpha$  homodimer formation, we investigated the retinoid-induced RXR homodimer binding to the TREP<sub>1</sub> using a gel retardation assay. Briefly, gel retardation assays were carried out

30 essentially as described previously<sup>33</sup>. *In vitro* translated receptor protein (1 to 5  $\mu$ l depending on the translation efficiency) was incubated with the <sup>32</sup>P-labeled oligonucleotides in a 20- $\mu$ l reaction mixture containing 10 mM Hepes buffer, pH 7.9, 50 mM KCl, 1 mM DTT, 2.5 mM MgCl<sub>2</sub>, 10% glycerol, and 1 mg of poly(dI-dC) at 25°C for 20

35 minutes. The reaction mixture was then loaded on a 5% nondenaturing polyacrylamide gel containing 0.5 x TBE (1 x TBE = 0.089 M Tris-borate, 0.089 M boric acid, and 0.002 M EDTA).

In the absence of *9-cis*-RA, RXR did not bind to this

response element. Retinoids SR11217, and SR11237 induced RXR homodimer binding to the response element in a concentration-dependent manner. Retinoid 11203, which behaved as a weak activator in the transient transfection assays, also induced only weak RXR binding.

5 SR11231 which did not activate the RXR homodimer was also not able to induce RXR homodimer binding. Similar results were obtained with the CRBPII-RARE and the ApoAI-RARE. We have thus defined here a class of synthetic retinoids that activate RXR $\alpha$  by inducing homodimer formation and binding to DNA.

10 An important question was whether these RXR-active compounds, like 9-cis-RA, would also activate RAR/RXR heterodimers or whether they would be truly RXR selective. To analyze this, we used four different reporter constructs carrying either the i) rat

15 cytoplasmic retinol binding protein I (CRBPI) gene RARE<sup>38</sup> that is only bound and activated by RAR/RXR heterodimers; ii) the RARB2 gene promoter RARE<sup>36,37</sup>, which is most effectively bound by heterodimers but also activated to some degree by RXR homodimers; iii) the CRBPII-RARE, which is activated only by RXR homodimers, and on which RAR represses

20 RXR activity<sup>30</sup>; iv) the apolipoprotein AI (apoAI) gene RARE<sup>31</sup> that is bound and activated by RAR/RXR heterodimers as well as by RXR homodimers. The four different reporter constructs were cotransfected with RAR $\alpha$ , RARB, RXR $\alpha$ , or with RXR $\alpha$  and RAR $\alpha$  together<sup>51</sup>. The retinoids were analyzed at a concentration of  $5 \times 10^{-7}$  M (a dose shown to yield

25 almost full induction (Fig. 7)).

CV-1 cells were cotransfected with 100 ng reporter plasmid a) CRBPI-tk-CAT, b) BRARE-tk-CAT, c) CRBPII-tk-CAT, and d) apoAI-tk-CAT. Retinoids were applied at  $5 \times 10^{-7}$  M. Results of a

30 representative experiment are shown.

The RXR-specific retinoids behaved strikingly different from 9-cis-RA (or RA) in that they only activated RXR homodimers but not RAR/RXR heterodimers. As with 9-cis-RA, both SR11217 and SR11237

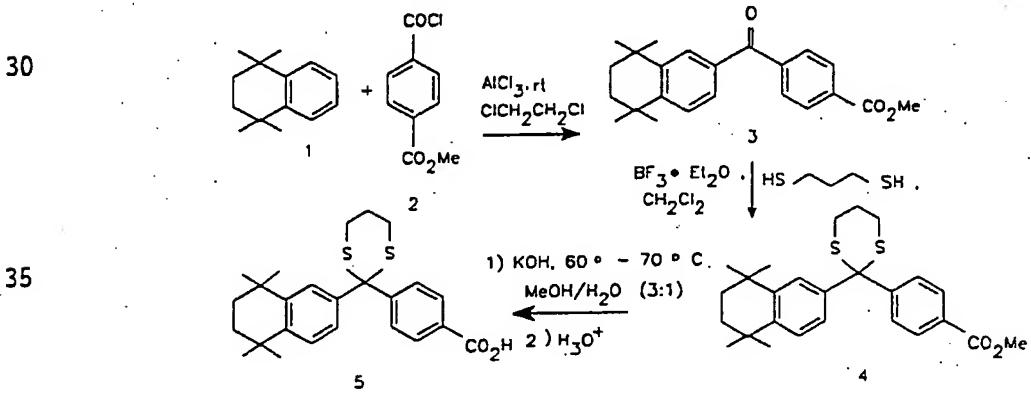
35 were strong activators of the CRBPII-RARE (i.e. the RARE that is significantly activated only by the RXR homodimer). However, in contrast to 9-cis-RA, they did not induce the CRBPI-RARE that is

activated only by the RAR/RXR heterodimer. Thus, while SR11217 and SR11237 behaved very similarly to 9-*cis*-RA on the CRBPII-RARE, they showed no response on the CRBPI-RARE, where 9-*cis*-RA is the optimal activator. The BRARE was slightly activated by SR11217 and SR11237, 5 consistent with the relatively low affinity of RXR homodimers for this response element. The apoAI-RARE was most effectively activated by RAR/RXR heterodimers in the presence of 9-*cis*-RA. In addition to the activity found in CV-1 cells, a significant and RXR-specific activation by retinoids SR11217 and SR11237 was also seen in various 10 other cell lines, including Hep G2 cells, where a particular high response was seen. RAR $\alpha$  and RAR $\beta$ , when cotransfected alone, were not activated significantly by any of the synthetic retinoids on any of the response elements tested. Similar negative results were obtained for RAR $\gamma$ . RAR $\alpha$  and  $\beta$  are assumed to form heterodimers with endogenous 15 RXR-like proteins in CV-1 cells, thus these heterodimers are also unresponsive to the synthetic retinoids.

Our data demonstrate that we have identified a novel class of retinoids that specifically induces RXR homodimer formation and that activates RXR homodimers on specific response elements but not 20 RAR/RXR heterodimers. These retinoids allow the specific activation of RXR-selective response pathways, while not inducing RAR-dependent response pathways. These retinoids provide a much more restricted physiological response than RA isomers or other retinoids presently used.

25

Example III



(a.) Methyl 4-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-5 naphthalenyl)carbonyl]benzoate (3):

To a suspension of aluminum chloride (1.13 g, 8.5 mmol) in 1.5 mL of 1,2-dichloroethane at 0°C under argon was added a solution of 1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene 1 (1.45 g, 7.7 mmol) (Kagechika, H., et al., J. Med. Chem. 31:2182 (1988)) and 4-carbomethoxybenzoyl chloride 2 (1.56 g, 7.9 mmol) (4-carbo-  
methoxybenzoyl chloride 2 was obtained from mono-methyl terephthalate, which is readily available from Aldrich, in one step (SOCl<sub>2</sub>, DMF)) in 6 mL of 1,2-dichloroethane. The resulting solution was brought to room temperature and stirred thereafter for 16 h. The reaction mixture was poured onto ice water and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with saturated aqueous NaHCO<sub>3</sub>, and brine. The solution was dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated to afford an orange solid (4.5 g). Flash chromatography (50% dichloromethane/hexane) yielded the desired product 3 as a pale yellow solid (2.07 g). Recrystallization from dichloromethane/hexane afforded the desired product 3 as a white, crystalline solid (1.96 g, 50%): m.p. 146-148°C; R<sub>f</sub> 0.14 (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

(b.) [2(5,6,7,8-Tetrahydro-5,5,8,8-tetramethylnaphthalen-2-yl)-2-(4-carbomethoxyphenyl)]-1,3-dithiane (4):

To a solution of the keto-ester 3 (97 mg, 0.277 mmol) in 3 mL of chloroform at 0°C under argon was added a solution of 1,3-propanedithiol (33  $\mu$ L, 36 mg, 0.332 mmol) followed by boron trifluoride etherate (17  $\mu$ L, 0.140 mmol). The resulting mixture was stirred at 0°C for 1 h and then warmed to room temperature overnight. The reaction mixture was quenched by pouring into saturated aqueous Na<sub>2</sub>CO<sub>3</sub>, and extracted with dichloromethane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to

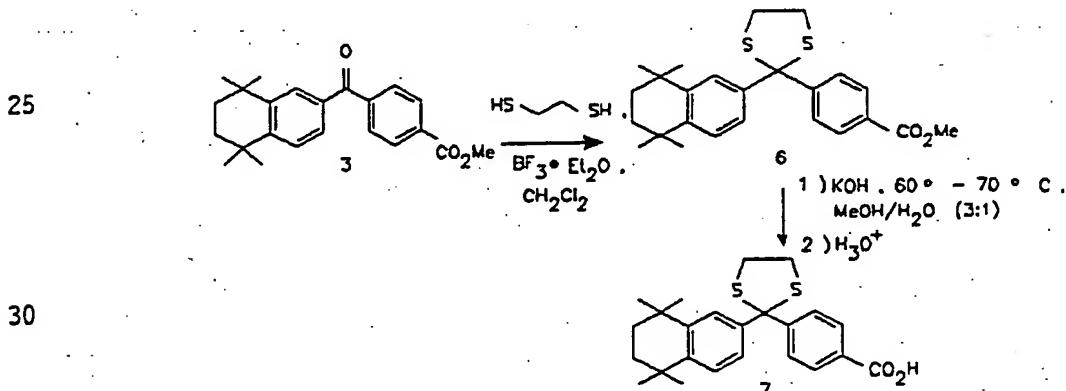
afford a yellow solid (0.108 g). Recrystallization from ethyl acetate/hexane afforded the desired dithiane 4 as a white, crystalline solid (0.087 g, 71%): m.p. 195-197°C; R, 0.32 (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

(c.) [2-(5,6,7,8-Tetrahydro-5,5,8-tetramethylnaphthalen-2-yl)-2-(4-carboxyphenyl)]-1,3-dithiane (5):

To a suspension of the ester 4 (85 mg, 0.193 mmol) in 10 75% aqueous methanol (2 mL) was added 0.024 g of potassium hydroxide and the mixture was stirred at 50°C for 2 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over 15 anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford a white solid. Recrystallization from benzene/hexane afforded the desired acid 5 as a white, powder (0.076 g, 92%): m.p. 229-231°C. The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

20

Example IV



(a.) [2-(5,6,7,8-Tetrahydro-5,5,8-tetramethyl-35 2-naphthalenyl)-2-(4-carbomethoxyphenyl)]-1,3-dithiolane (6):

To a solution of the keto-ester 3 (80 mg, 0.228 mmol) in dichloromethane (2 mL) at 0°C under argon was added a solution of

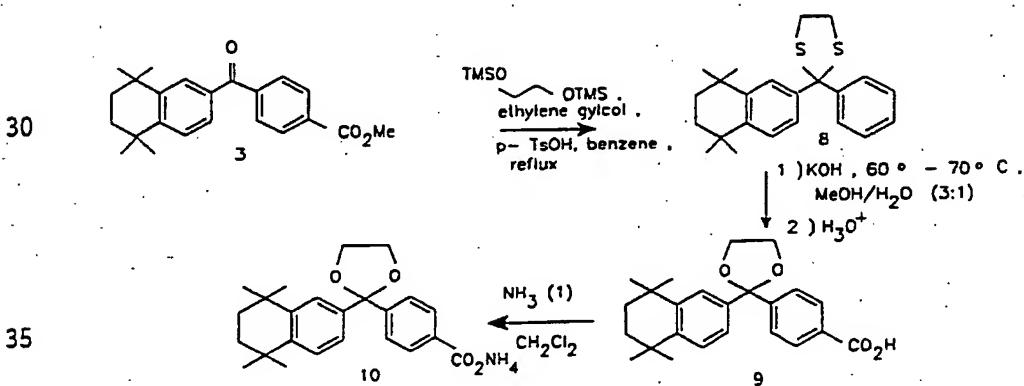
ethanedithiol (26 mg, 0.27 mmol) in dichloromethane (0.5 mL) followed by boron trifluoride etherate (0.04 mL, 0.3 mmol). The resulting mixture was stirred at 0°C for 1 h and then warmed to room temperature overnight. The reaction mixture was quenched by pouring into 5 saturated aqueous Na<sub>2</sub>CO<sub>3</sub>, and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford a solid. Flash chromatography (30; 40% CH<sub>2</sub>Cl<sub>2</sub>/hexane) yielded the desired dithiane 6 as a white solid (0.088 g, 90%): m.p. 105-107°C; R, 0.33 (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane). The structure of 10 the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

(b.) [2-(5,6,7,8-Tetrahydro-5,5,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)]-1,3-dithiolane (7):

To a suspension of the ester 6 (85 mg, 0.199 mmol) in 75% aqueous methanol (3 mL) was added one pellet of potassium 15 hydroxide (0.11 g) and the mixture was stirred at 70°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers when dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford 20 a white solid. Recrystallization from dichloromethane-hexane afforded the desired acid 7 as a white powder (0.064 g, 79%): m.p. 218-221°C. The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

25

Example V

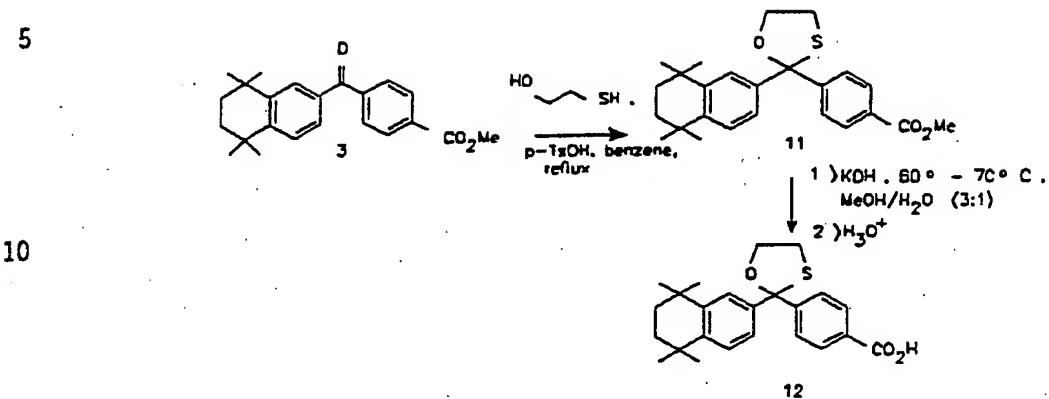


(a.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carbomethoxyphenyl)]-1,3-dioxolane (8):

To a solution of keto-ester 3 (80 mg, 0.228) in 1 mL of benzene was added ethylene glycol (1 mL), 1,2-bis(trimethylsilyloxy)ethane (2 mL) and a catalytic amount of *p*-TsOH. The reaction mixture was heated at reflux for 4 h and then cooled to room temperature. The solution was poured into saturated aqueous NaHCO<sub>3</sub> and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford a solid. Flash chromatography (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane) yielded the desired ketal 8 as a white solid (0.082 g, 91%): m.p. 145-147°C; R<sub>f</sub> 0.16 (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

15. (b.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)]-1,3-dioxolane ammonium salt (10):

To a suspension of the ester 8 (50 mg, 0.127 mmol) in 75% aqueous methanol (2 mL) was added one pellet of potassium hydroxide (0.1 g), and the reaction mixture was stirred at 70°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford a white solid 9. The acid 9 was dissolved in dichloromethane (4 mL) under argon, and ammonia gas was condensed into the solution; which was stirred for 5 min at -33°C. The solution was warmed to room temperature for 20 min to evaporate the ammonia and concentrated to afford the ammonium salt 10 as a white powder (47 mg, 93%): m.p. 259-261°C. The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

Example VI

(a.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carbomethoxyphenyl)]-1,3-oxathiolane (11):

To a solution of keto-ester 3 (88 mg, 0.251) in 2 ml of benzene was added 2-mercaptoethanol (1 mL), and a catalytic amount of p-TsOH. The reaction mixture was heated at reflux overnight and then cooled to room temperature. The solution was poured into saturated aqueous NaHCO<sub>3</sub>, and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford a solid. Flash chromatography (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane) yielded the desired ketal 11 as a white solid (0.09 g, 87%): m.p. 122-124°C; R<sub>f</sub> 0.24 (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

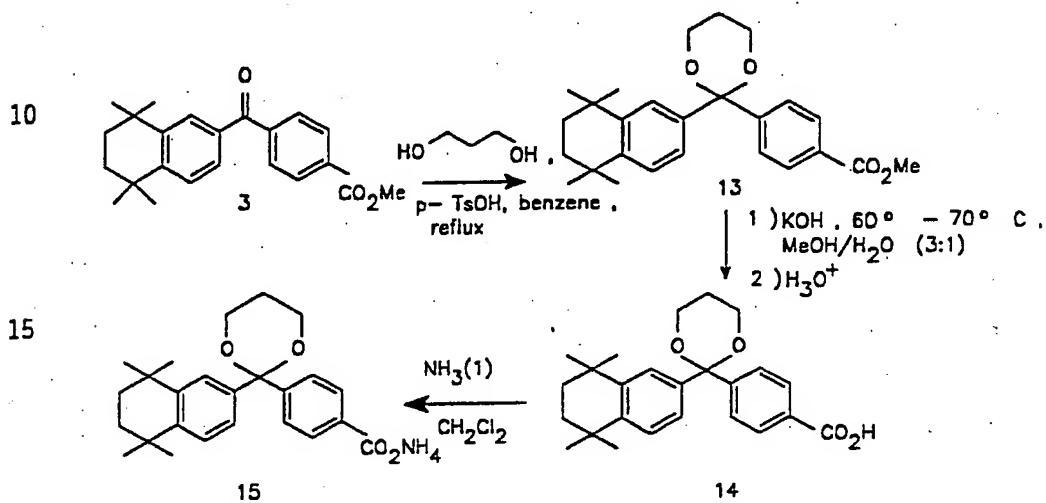
(b.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)]-1,3-oxathiolane (12):

To a suspension of the ester 11 (64 mg, 0.156 mmol) in 75% aqueous methanol (3 mL) was added one pellet of potassium hydroxide (0.12 g), and the reaction mixture was stirred at 75°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford

a white solid. Recrystallization from dichloromethane-hexane afford the desired acid 12 as a white powder (0.06 g, 97%): m.p. 216-217.5°C. The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

5

Example VII



20

(a.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carbomethoxyphenyl)]-1,3-dioxane (13):

To a solution of keto-ester 3 (150 mg, 0.428 mmol) in 5 ml of benzene was added 1,3-propanediol (1.5 mL), and a catalytic amount of p-TsOH. The reaction mixture was heated at reflux overnight and then cooled to room temperature. The solution was poured into saturated aqueous NaHCO<sub>3</sub>, and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford a solid. Flash chromatography (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane) yielded the desired ketal 13 as a white solid (0.164 g, 94%): m.p. 157-159°C; R<sub>f</sub> 0.24 (5% ethyl acetate/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

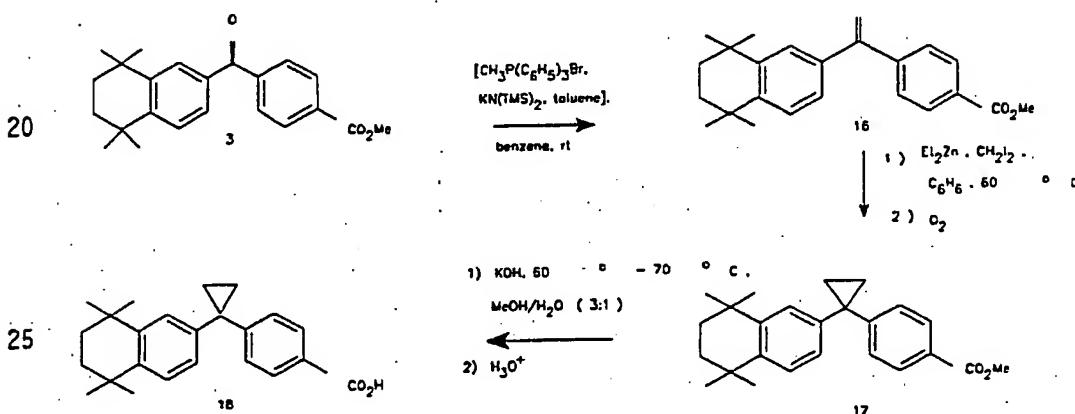
35

(b.) [2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-(4-carboxyphenyl)]-1,3-dioxane ammonium salt (15):

To a suspension of the ester 13 (0.1 g, 0.245 mmol) in

75% aqueous methanol (3 mL) was added one pellet of potassium hydroxide (0.12 g). The reaction mixture was stirred at 80°C for 30 min during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and 5 then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous  $\text{MgSO}_4$ , filtered, and concentrated to afford a white solid 14. The acid 14 was dissolved in dichloromethane (4 mL) under argon. Ammonia gas was condensed into the flask and the mixture was stirred for 5 min at -33°C. The solution was warmed to 10 room temperature for 20 min to evaporate ammonia and concentrated to afford the ammonium salt 15 as a white powder (0.238 g, 97%): m.p. 228-230°C. The structure of the product was also confirmed using IR,  $^1\text{H}$  NMR and mass spectroscopy.

15

Example VIII

(a.) Methyl 4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-30 2-naphthalenyl)-1-ethenyl]benzoate (16):

To a suspension of methyltriphenylphosphonium bromide (0.78 g, 2.18 mmol) in 5 mL of benzene under argon at room temperature was added a 0.5 M solution of potassium hexamethyldisilazide in toluene (4.4 mL, 2.2 mmol), and the yellow solution was stirred for 5 min. A solution of keto-ester 3 (0.51 g, 1.455 mmol) in 7 mL of benzene was added, and the orange solution was stirred for 1 h at room temperature. The reaction mixture was poured into saturated aqueous

NaHCO<sub>3</sub> and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered through a plug of silica gel, and concentrated to afford a solid. Flash chromatography (30% dichloromethane/hexane) yielded the desired 5 product 16 as a white solid (0.405 g, 80%): m.p. 117-118°C; R<sub>f</sub> 0.2 (25% CH<sub>2</sub>Cl<sub>2</sub>/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

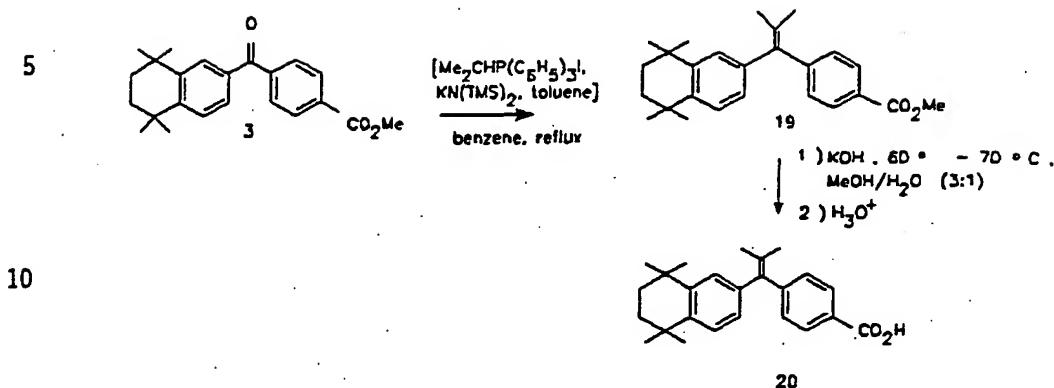
(b.) [1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-10 2-naphthalenyl)-1-(4-carbomethoxyphenyl)]-cyclopropane (17):

To a solution of ethene 16 (0.130 g, 0.373 mmol) in 10 mL of benzene under argon at room temperature was added a 1 M solution of diethylzinc in hexane (5.6 mL, 5.6 mmol), and the reaction mixture was heated to 60°C. Diiodomethane (0.48 mL, 6.0 mmol) in 2 mL of benzene 15 was added dropwise for 5 min. The reaction mixture was cooled to room temperature and oxygen was bubbled through for 3 h. The cloudy solution was diluted with 40% ethyl acetate/hexane and washed with aqueous hydrochloric acid, water and saturated aqueous NaHCO<sub>3</sub>. The organic layer was dried over anhydrous MgSO<sub>4</sub>, filtered, and 20 concentrated to afford a solid. Flash chromatography (30%; 40% CH<sub>2</sub>Cl<sub>2</sub>/hexane) yielded the desired product 17 as a white solid (0.08 g, 59%): m.p. 100-102°C; R<sub>f</sub> 0.36 (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

25 (c.) [1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-(4-carboxyphenyl)]cyclopropane (18):

To a suspension of the ester 17 (60 mg, 0.166 mmol) in 75% aqueous methanol (2 mL) was added one pellet of potassium hydroxide (0.12 g), and the reaction mixture was stirred at 70°C for 1 30 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford a white solid. Recrystallization from dichloromethane-hexane 35 afforded the desired acid 18 as a white powder (0.055 g, 95%): m.p. 333-335°C. The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

### Example IX



15 (a.) Methyl 4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-methyl-1-propenyl]benzoate (19):

To a suspension of isopropyltriphenylphosphonium iodide (0.35 g, 0.807 mmol) in 3 mL of benzene under argon at room temperature was added a 0.5 M solution of potassium hexamethyl-disilazide in toluene (1.8 mL, 0.89 mmol), and the red solution was stirred for 5 min. A solution of keto-ester 3 (0.169 g, 0.481 mmol) in 3 mL of benzene was added, and the red solution was heated to 110°C, while approximately 4 mL of benzene was distilled out. After 1 h, the reaction mixture was diluted with 40% ethyl acetate/hexane and washed with saturated aqueous NaHCO<sub>3</sub> and brine. The organic layer was dried over anhydrous MgSO<sub>4</sub>, filtered through a plug of silica gel, and concentrated to afford a solid. Flash chromatography (40% dichloromethane/hexane) yielded the desired product 19 as a white powder (0.128 g, 71%): R<sub>f</sub> 0.44 (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

(b.) 4-[1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-methyl-1-propenyl]benzoic acid (20):

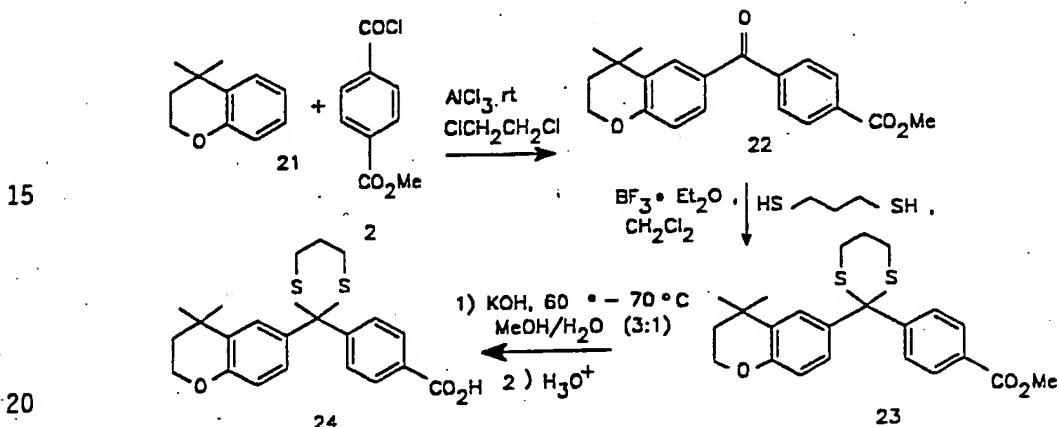
To a suspension of the ester 19 (0.115 g, 0.304 mmol) in 35 75% aqueous methanol (3 mL) was added one pellet of potassium hydroxide (0.12 g). The mixture was stirred at 75°C for 1 h during which time the material dissolved. The solution was cooled to room

temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous  $MgSO_4$ , filtered, and concentrated to afford the desired acid 20 as a white powder (0.11 g, 99%): m.p. 204-206°C.

5 The structure of the product was also confirmed using IR,  $^1H$  NMR and mass spectroscopy.

Example X

10



(a.) Methyl 4-[(4,4-dimethyl-3,4-dihydro-2H-1-benzopyran-6-yl)carbonyl]benzoate (22) (Maignan, J., et al., BE 1,000,195):

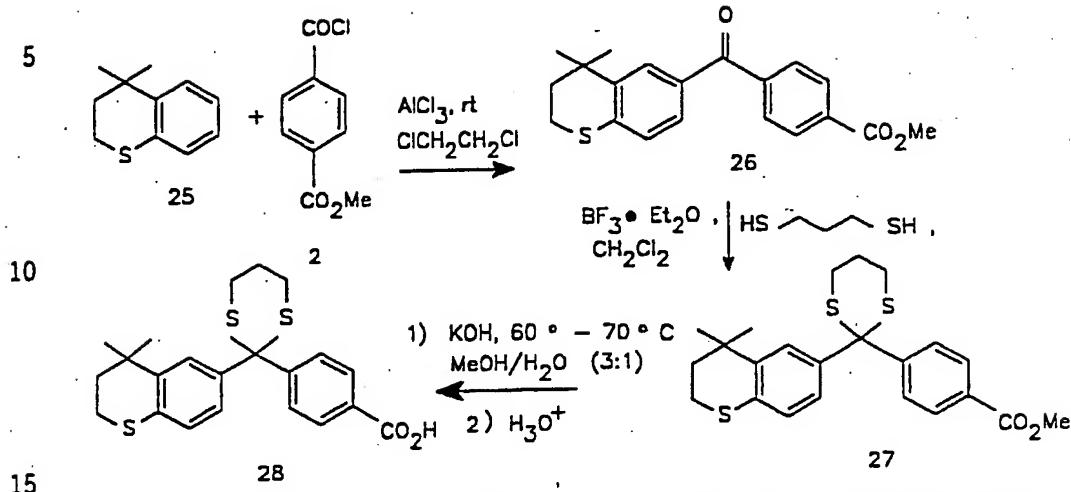
To a suspension of aluminum chloride (1.6 g, 12 mmol) in 25 1 mL of 1,2-dichloroethane under argon at room temperature was added a solution of 4,4-dimethyl-3,4-dihydro-2H-1-benzopyran 21 (1.5 g, 9.25 mmol) (Dawson, M.I., et al., J. Med. Chem. 27:1516-1531 (1984)) and 4-carbomethoxybenzoyl chloride 2 (1.79 g, 9 mmol) in 9 mL of 1,2-dichloroethane. The reaction mixture was stirred overnight, 30 poured onto ice-water and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with saturated aqueous  $NaHCO_3$ , and brine. The solution was dried over anhydrous  $MgSO_4$ , filtered, and concentrated to afford a yellow solid (3.24 g). Flash chromatography (80% dichloromethane/hexane) yielded the desired product 22 as a white 35 powder (1.42 g, 49%): m.p. 129-131°C;  $R_f$  0.26 ( $CH_2Cl_2$ ). The structure of the product was also confirmed using IR,  $^1H$  NMR and mass spectroscopy.

(b.) [2-(4,4-Dimethyl-3,4-dihydro-2H-1-benzopyran-6-yl)-2-(4-carbomethoxyphenyl)]-1,3-dithiane (23):

To a solution of the keto-ester 22 (0.152 g, 0.469 mmol) in dichloromethane (3 mL) at 0°C under argon was added 1,3-propane-5-dithiol (0.061 g, 0.563 mmol), followed by boron trifluoride etherate (0.07 mL, 0.57 mmol). The resulting mixture was stirred at 0°C for 1 h and at ambient temperature overnight. The reaction mixture was quenched by pouring into saturated aqueous Na<sub>2</sub>CO<sub>3</sub>, and then extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford an oil. Flash chromatography (80% dichloromethane/hexane) yielded the desired dithiane 23 as a white solid (0.175 g, 90%): m.p. 103-105°C; R<sub>f</sub> 0.2 (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

(c.) [2-(4,4-Dimethyl-3,4-dihydro-2H-1-benzopyran-6-yl)-2-(4-carboxyphenyl)]-1,3-dithiane (24):

To a suspension of the dithiane 23 (0.145 g, 0.349 mmol) in 75% aqueous methanol (4 mL) was added one pellet of potassium hydroxide (0.106 g). The reaction mixture was stirred at 70°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford a white solid. Recrystallization from dichloromethane-hexane afforded the desired acid 24 as a white powder (0.127 g, 90%): m.p. 204-205°C. The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

Example XI

(a.) Methyl 4-[(4,4-dimethyl-3,4-dihydro-2H-1-benzothiopyran-6-yl)carbonyl]benzoate (**26**):

To a suspension of aluminum chloride (1.65 g, 9.25 mmol) in 1 mL of 1,2-dichloroethane under argon at room temperature was added a solution of 4,4-dimethyl-3,4-dihydro-2H-1-benzothiopyran **25** (1.65 g, 9.25 mmol) (Waugh, K.M., et al., *J. Med. Chem.* **28**:116-124 (1985)) and 4-carbomethoxybenzoyl chloride **2** (1.8 g, 9.06 mmol) in 9 mL of 1,2-dichloroethane. The reaction mixture was stirred overnight and poured onto ice water and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with saturated aqueous  $\text{NaHCO}_3$  and brine. The solution was dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated to afford a green solid (2.1 g). Flash chromatography (80% dichloromethane/hexane) yielded the desired product **26** as a white powder (1.23 g, 40%): m.p. 118-120°C;  $R_f$  0.35 ( $\text{CH}_2\text{Cl}_2$ ). The structure of the product was also confirmed using IR,  $^1\text{H}$  NMR and mass spectroscopy.

(b.) [2-(4,4-Dimethyl-3,4-dihydro-2H-1-benzothiopyran-6-yl)-2-(4-carbomethoxyphenyl)]-1,3-dithiane (**27**):

To a solution of the keto-ester **26** (0.12 g, 0.353 mmol) in dichloromethane (3 mL) at 0°C under argon was added 1,3-propandithiol (0.06 mL, 0.53 mmol) followed by boron trifluoride etherate (0.07 mL,

0.57 mmol). The resulting mixture was stirred at 0°C for 1 h and then warmed to room temperature overnight. The reaction mixture was quenched by pouring into saturated aqueous  $\text{Na}_2\text{CO}_3$ , and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous  $\text{MgSO}_4$ , filtered, and concentrated to afford an oil. Flash chromatography (80%  $\text{CH}_2\text{Cl}_2$ /hexane) yielded the desired dithiane 27 as a white solid (0.145 g, 96%): m.p. 164-166°C;  $R_f$  0.27 (50%  $\text{CH}_2\text{Cl}_2$ /hexane). The structure of the product was also confirmed using IR,  $^1\text{H}$  NMR and mass spectroscopy.

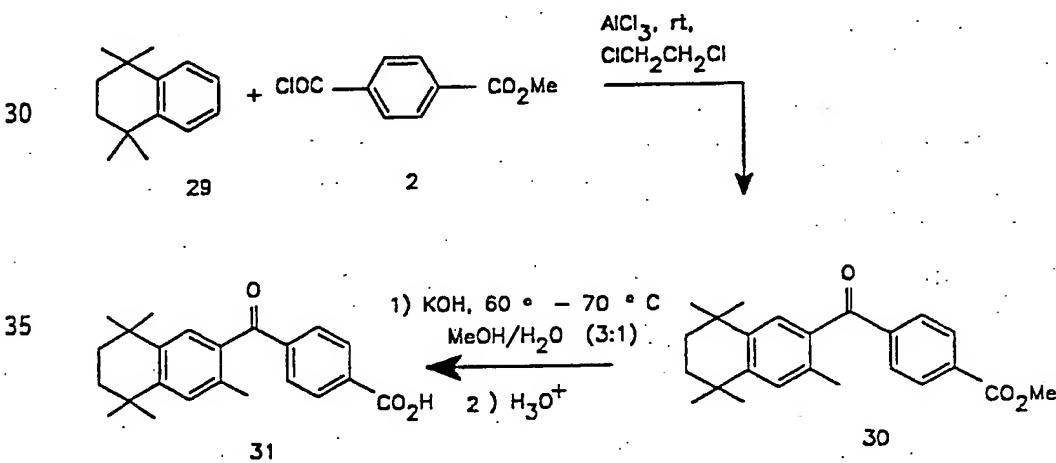
10

(c.) [2-(4,4-Dimethyl-3,4-dihydro-2H-1-benzothiopyran-6-yl)-2-(4-carboxyphenyl)]-1,3-dithiane (28):

To a suspension of the dithiane 27 (0.1 g, 0.232 mmol) in 75% aqueous methanol (4 mL) was added one pellet of potassium hydroxide (0.12 g). The reaction mixture was stirred at 75°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous  $\text{MgSO}_4$ , filtered, and concentrated to afford a white solid. Recrystallization from dichloromethane/hexane afforded the desired acid 28 as a white powder (0.089 g, 92%): m.p. 211-212.5°C. The structure of the product was also confirmed using IR,  $^1\text{H}$  NMR and mass spectroscopy.

25

Example XII

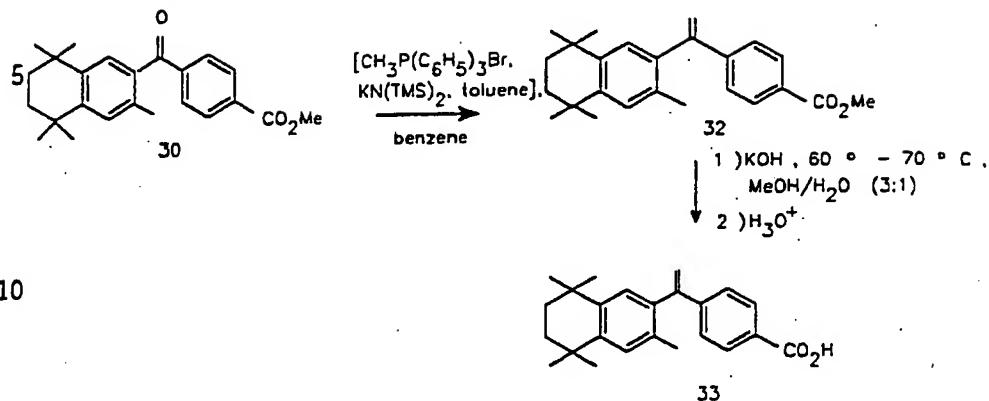


(a.) Methyl [4-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)carbonyl]benzoate (30):

To a suspension of aluminum chloride (1.10 g, 8.25 mmol) in 30 mL of 1,2-dichloroethane under argon at room temperature was 5 added a solution of 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethyl-naphthalene 29 (1.52 g, 7.5 mmol) (Kagechika, H., et al., J. Med. Chem. 31:2182 (1988)) and 4-carbomethoxybenzoyl chloride 2 (1.57 g, 7.87 mmol) in 15 mL of 1,2-dichloroethane. The reaction mixture was 10 stirred overnight and poured onto ice water and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with saturated aqueous NaHCO<sub>3</sub> and brine. The solution was dried over anhydrous MgSO<sub>4</sub>, filtered and concentrated to afford a brown solid (2.56 g). Flash chromatography (60% dichloromethane/hexane) yielded 15 the desired product 30 as a white, crystalline solid (1.733 g, 64%): m.p. 146-149°C; R<sub>f</sub> 0.11 (50% CH<sub>2</sub>Cl<sub>2</sub>/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

(b.) [4-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)carbonyl]benzoic acid (31):

To a suspension of the ester 30 (0.120 g, 0.329 mmol) in 20 75% aqueous methanol (2 mL) was added potassium hydroxide (0.055 g). The reaction mixture was stirred at 60°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 25 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford a white solid (0.109 g). Recrystallization from benzene/hexane afforded 31 as a 30 white, crystalline solid (0.102 g, 89%): m.p. 209-212°C. The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

Example XIII

15 (a.) Methyl 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)-1-ethenyl]benzoate (32):

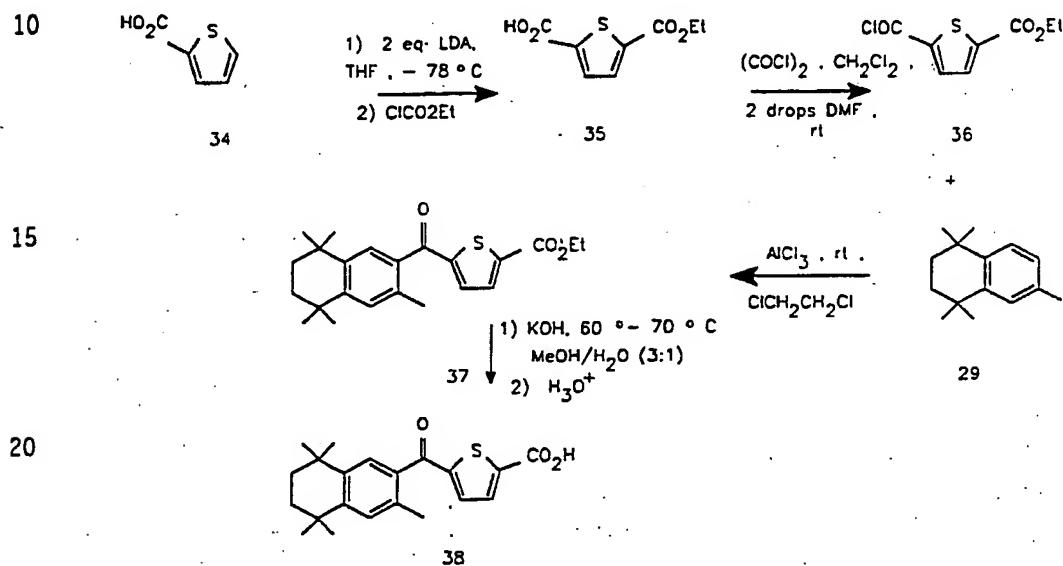
To a suspension of methyltriphenylphosphonium bromide (0.196 g, 0.55 mmol) in 1 mL of benzene under argon at room temperature was added a 0.5 M solution of potassium hexamethyldisilazide in toluene (1.2 mL, 0.6 mmol), and the yellow solution was stirred for 5 min. A solution of keto-ester 30 (0.1 g, 0.274 mmol) in 1.5 mL of benzene was added, and the orange solution was stirred for 3 h at room temperature. The reaction mixture was filtered through a plug of silica gel with 40% ethyl acetate/hexane. The filtrate was concentrated to afford a solid. Flash chromatography (30%; 40% dichloromethane/ hexane) yielded the desired product 32 as a white solid (0.077 g, 78%): m.p. 167-168°C; R<sub>f</sub> 0.4 (50% dichloromethane/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

30 (b.) [4-[1-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)-1-ethenyl]benzoic acid (33):

To a suspension of the ester 32 (0.058 g, 0.16 mmol) in 75% aqueous methanol (2 mL) was added one pellet of potassium hydroxide (0.1 g). The mixture was stirred at 70°C for 1 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then

extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous  $MgSO_4$ , filtered, and concentrated to afford a white solid. Recrystallization from dichloromethane/hexane afforded the desired acid 33 as a white, crystalline solid (42 mg, 91%): m.p. 5 230-231°C. The structure of the product was also confirmed using IR,  $^1H$  NMR and mass spectroscopy.

Example XIV



25 (a.) 5-Carboethoxythiophen-2-carboxylic acid (35):

To a solution of diisopropylamine (3.6 mL, 25.75 mmol) in 10 mL of THF at -78°C under argon was added a 1.6 M solution of  $n$ -butyllithium in hexane (16.1 mL, 25.75 mmol). The mixture was stirred for 15 min, and a solution of 2-thiophenecarboxylic acid 34 (1.5 g, 11.705 mmol) (2-thiophenecarboxylic acid 34 is readily available from Aldrich) in 5 mL of THF was added slowly. The mixture was stirred for 15 min, and ethyl chloroformate (2.7 mL, 28.33 mmol) was added. The mixture was stirred for 30 min at -78°C, and warmed to 0°C for another 30 min. The reaction mixture was poured into saturated aqueous  $NaHCO_3$ , and washed with 80% ethyl acetate/hexane. The aqueous layer was acidified with acetic acid and extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over

anhydrous  $MgSO_4$ , filtered, and concentrated to afford a yellow solid. Flash chromatography yielded the desired acid 35 (1.76 g, 75%) as a white solid: m.p.  $>300^\circ C$ . The structure of the product was also confirmed using IR,  $^1H$  NMR and mass spectroscopy.

5

(b.) 5-Carboethoxythiophen-2-carbonyl chloride (36):

To a suspension of acid 35 (0.64 g, 3.2 mmol) in dichloromethane (20 mL) under argon at room temperature was added a 2.0 M solution of  $(COCl)_2$  in dichloromethane (2.4 mL, 4.8 mmol) and two drops of DMF, 10 and stirred overnight. Excess  $(COCl)_2$  and dichloromethane were removed at reduced pressure, and the viscous, light, yellow product was dried overnight to afford the desired benzoyl chloride 36 as a yellow solid. The structure of the product was also confirmed using IR and  $^1H$  NMR spectroscopy.

15

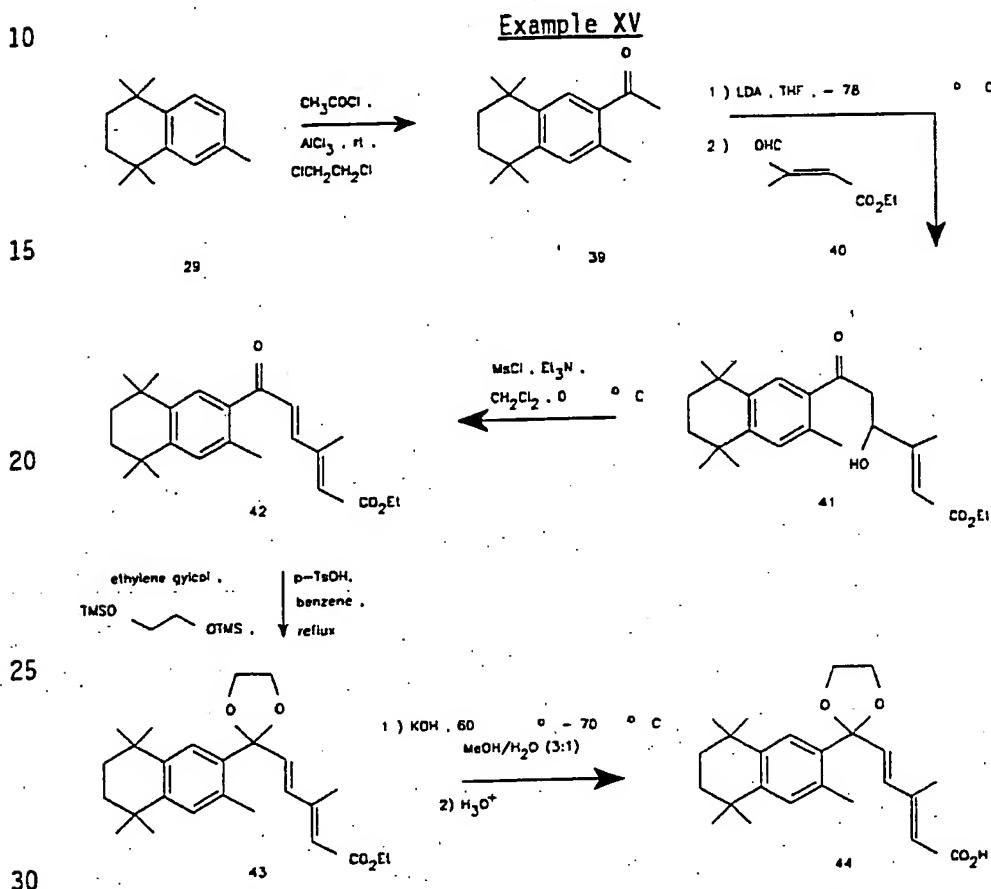
(c.) Ethyl 5-[(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)carbonyl]thiophen-2-carboxylate (37):

To a suspension of aluminum chloride (0.5 g, 3.75 mmol) in 1 mL of 1,2-dichloroethane under argon at room temperature was added a 20 solution of 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethylnaphthalene 29 (0.712 g, 3.52 mmol) and 36 (0.7 g, 3.2 mmol) in 3.5 mL of 1,2-dichloroethane dropwise. The reaction mixture was stirred overnight, and then poured onto ice water, and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with 25 saturated aqueous  $NaHCO_3$  and brine. The solution was dried over anhydrous  $MgSO_4$ , filtered and concentrated to afford a yellow solid (1.5 g). Flash chromatography (50% dichloromethane/hexane) yielded a light-yellow solid. Recrystallization from dichloromethane/hexane afforded 37 as a white crystalline solid (0.62 g, 50%): m.p. 30 111-112°C; R, 0.2 (50%  $CH_2Cl_2$ /hexane). The structure of the product was also confirmed using IR,  $^1H$  NMR and mass spectroscopy.

(d.) 5-[(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)carbonyl]thiophen-2-carboxylic acid (38):

35 To a suspension of the ester 37 (50 mg, 0.13 mmol) in 75% aqueous methanol (3 mL) was added one pellet of potassium hydroxide (0.1 g). The reaction mixture was stirred at 70°C for 1.5 h during

which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous  $MgSO_4$ , filtered, and concentrated to afford 5 a white solid (46 mg). Recrystallization from methanol afforded the desired acid 38 as a white crystalline solid (42 mg, 91%): m.p. 214-215°C. The structure of the product was also confirmed using IR,  $^1H$  NMR and mass spectroscopy.



(a.) 2-Acetyl-3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalene (39):

To a suspension of aluminum chloride (0.96 g, 7.17 mmol) in 1 mL of 1,2-dichloroethane under argon at room temperature was 35 added a solution of 1,2,3,4-tetrahydro-1,1,4,4,6-pentamethyl-naphthalene 29 (1.2 g, 5.93 mmol) and acetyl chloride (0.51 g, 6.52 mmol) in 9 mL of 1,2-dichloroethane. The reaction mixture was stirred

for 1 h and then was poured onto ice water and extracted with 40% ethyl acetate/hexane. The combined organic layers were washed with saturated aqueous  $\text{NaHCO}_3$ , and brine. The solution was dried over anhydrous  $\text{MgSO}_4$ , filtered through a plug of silica gel, and 5 concentrated to afford a white solid 39 (1.45 g, 99%): m.p. 54-57°C;  $R_f$  0.62 (10% ethyl acetate/hexane). The structure of the product was also confirmed using IR,  $^1\text{H}$  NMR and mass spectroscopy.

(b.) Ethyl  $(E)$ -4-hydroxy-3-methyl-6-oxo-6-(3,5,5,8,8-  
10 pentamethyl-5,6,7,8-tetrahydro-2-naphthalenyl)-2-hexenoate (41):

To a solution of diisopropylamine (0.5 mL, 3.52 mmol) in 7 mL of THF at -78°C under argon was added a 1.6 M solution of  $n$ -butyllithium in hexane (2.2 mL, 3.52 mmol). The mixture was stirred for 25 min, and a solution of ketone 39 (0.78 g, 3.2 mmol) in 4 mL of 15 THF was added slowly. The mixture was stirred for 20 min, and a solution of ethyl  $(E)$ -3-formyl-2-butenoate 40 (0.45 g, 3.2 mmol) (ethyl  $(E)$ -3-formyl-2-butenoate 40 is readily available from Fluka) in 3 mL of THF was added slowly. The mixture was stirred for 35 min at -78°C, poured into saturated aqueous  $\text{NH}_4\text{Cl}$ , and extracted with 40% 20 ethyl acetate/hexane. The combined organic layers were dried over anhydrous  $\text{MgSO}_4$ , filtered, and concentrated to afford a light-yellow solid. Flash chromatography yielded the desired alcohol 41 as a white crystalline solid (0.98 g, 80%): m.p. 126-128°C;  $R_f$  0.13 (10% ethyl acetate/hexane). The structure of the product was also confirmed 25 using IR,  $^1\text{H}$  NMR and mass spectroscopy.

(c.) Ethyl  $(2E,4E)$ -6-oxo-6-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2- naphthalenyl)-2,4-hexadienoate (42):

To a solution of 41 (0.33 g, 0.85 mmol) in 8 mL of THF at 30 0°C was added triethylamine (0.5 mL, 4 mmol), and a solution of methylsulfonyl chloride (0.106 g, 0.93 mmol) in 2 mL of THF slowly. The mixture was stirred for 1 h at 0°C and warmed to room temperature for 30 min. The mixture was filtered through a plug of silica gel with 10% ethyl acetate/hexane. The filtrate was concentrated to 35 afford 42 as a light-yellow solid. Recrystallization from dichloromethane/hexane afforded the desired acid 42 as a yellow powder (0.3 g, 95%): m.p. 101-102°C;  $R_f$  0.42 (10% ethyl acetate/hexane). The

structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

(d.) [2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)-2-(4-carboethoxy-2E,4E-3-methylbutadienyl)]-1,3-dioxolane (43):

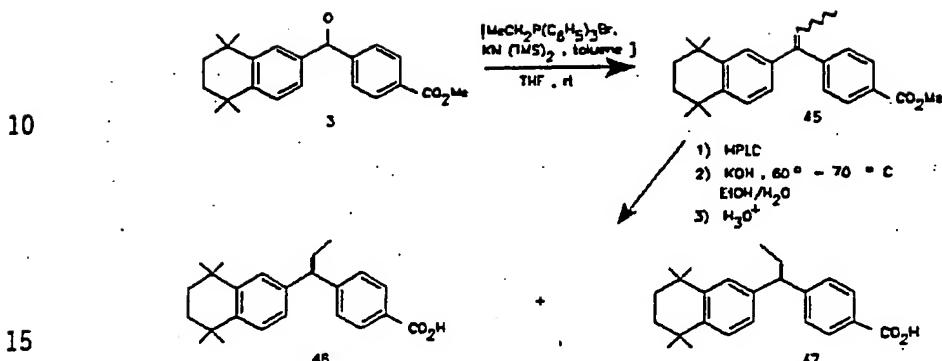
To a solution of keto-ester 42 (0.12 g, 0.33) in 2 mL of benzene was added ethylene glycol (0.8 mL), 1,2-bis(trimethylsilyloxy)ethane (2 mL) and a catalytic amount of p-TsOH. The reaction mixture was 10 heated at reflux for 2 days and then cooled to room temperature. The solution was poured into saturated aqueous NaHCO<sub>3</sub> and extracted with 40% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford a solid. Flash chromatography (5% ethyl acetate/hexane) yielded the desired ketal 43 15 as a colorless oil (0.109 g, 80 %): R<sub>f</sub> 0.43 (10% ethyl acetate/hexane). The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

(e.) [2-(5,6,7,8-Tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)-2-(4-carboxy-2E,4E-3-methylbutadienyl)]-1,3-dioxolane (44):

To a solution of the ester 43 (30 mg, 0.073 mmol) in 75% aqueous methanol (2 mL) was added one pellet of potassium hydroxide (0.1 g). The reaction mixture was stirred at 60°C for 0.5 h 25 during which time the material dissolved. The solution was cooled to room temperature, acidified with 1 N aqueous hydrochloric acid, and then extracted with 80% ethyl acetate/hexane. The combined organic layers were dried over anhydrous MgSO<sub>4</sub>, filtered, and concentrated to afford a white solid. Recrystallization from dichloromethane/hexane 30 afforded the desired acid 44 as a white, crystalline solid (27 mg, 96 %): m.p. 189-190°C. The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

Example XVI

5



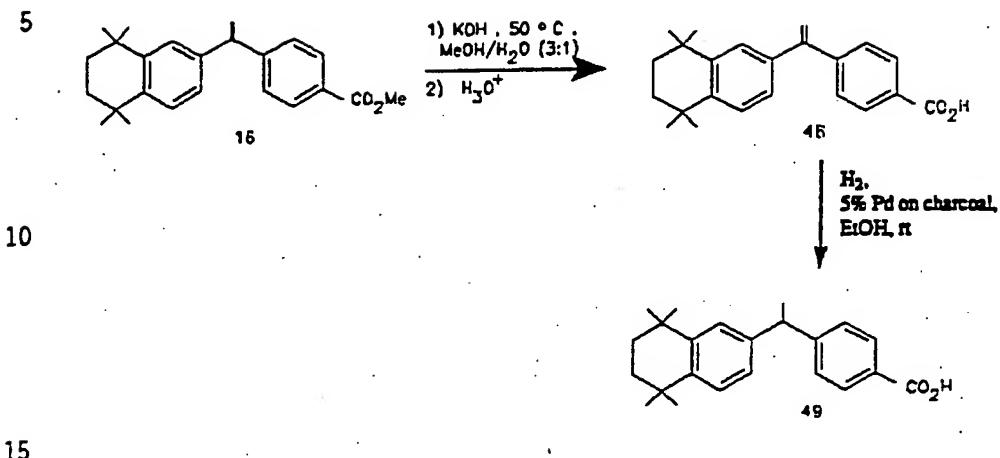
20 (E) and (Z)-4-[1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)propen-1-yl]benzoic acid (46 and 47):

To a suspension of ethyltriphenylphosphonium bromide (160 mg, 0.43 mmol) in 1 mL of THF under argon at 0°C was added a 0.5 M solution of potassium hexamethyldisilazide in toluene (0.95 mL, 0.47 mmol), and the reaction mixture was stirred for 5 min. A solution of 25 keto-ester 3 (100 mg, 0.28 mmol) in 0.8 mL of THF was added, and the orange solution was stirred at room temperature for 3 h. The reaction mixture was diluted with 20% ethyl acetate/hexane and filtered through a plug of silica gel with 20% ethyl acetate/hexane. The solution was concentrated to afford a yellow gum. Chromatography (38% dichloromethane/hexane) yielded the mixture of 45 as a pale-yellow gum (51 mg, 50%);  $R_f$  0.43, 0.47 (40%  $CH_2Cl_2$ /hexane). Preparative HPLC (Waters Radialpak Novapak silica, 8 mm x 10 cm, 2% ether/hexane, 1.0 mL/min, 260 nm) gave a colorless gum 45a (20 mg,  $t_r$  = 9.8 min) and a white solid 45b (25 mg,  $t_r$  = 10.8 min).

To a suspension of the ester 45a (20 mg) in 0.5 mL of ethanol was added a 40% solution of aqueous potassium hydroxide (0.2 g), and the reaction mixture was stirred at 70°C under argon for 2 h during which time the material dissolved. The solution was then 5 concentrated in an argon stream. The residue was cooled to room temperature, acidified with 1 N aqueous sulfuric acid to pH 2-3, and then filtered. The precipitate was repeatedly washed with water (6 x 1 mL) and dried to afford a white powder (20 mg). Recrystallization from ethyl acetate/hexane afforded the acid 46 as a 10 white powder (16 mg, 16% overall yield): m.p. 212°C. The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy. The regiochemistry of 46 was confirmed by <sup>1</sup>H nOe NMR spectroscopy.

The ester 45b was hydrolyzed as above to give 25 mg of 15 pale-yellow solid. Recrystallization from ethyl acetate afforded the acid 47 as a pale-yellow powder (21 mg, 20% overall yield): m.p. 229°C. The structure of the product was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy. The regiochemistry of 47 was confirmed by <sup>1</sup>H nOe NMR spectroscopy.

### Example XVII



(a.) 4-[1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-ethenyl]benzoic acid (48):

20 To a suspension of the ester 16 (94 mg, 0.27 mmol) in 75% aqueous methanol (2 mL) was added potassium hydroxide (0.045 g), and the reaction mixture was stirred at 50°C for 14 h during which time the material dissolved. The solution was cooled to room temperature, acidified with 2 N aqueous hydrochloric acid, and then extracted with ether. The combined organic layers were washed with water and brine. The organic solution was then dried over anhydrous  $\text{MgSO}_4$ , filtered and concentrated to afford a white solid. Recrystallization from benzene-hexane afforded the desired acid 48 as a white crystalline solid (0.074 g, 82%): m.p. 201-204°C. The structure of the product was 25 30 also confirmed using IR,  $^1\text{H}$  NMR and mass spectroscopy.

(b.) 4-[1-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-ethyl]benzoic acid (49):

The olefin 48 (0.0105 g, 0.0314 mmol) was hydrogenated over 5% palladium on charcoal (1 mg) in 0.5 mL of ethanol at room temperature and atmospheric pressure. After one equivalent (0.7 mL) of hydrogen was taken up, the catalyst was removed by filtration

through a small Celite pad. The solvent was removed in vacuo to give the crude acid as a white solid (0.019 g). Recrystallization from benzene-hexane afforded the desired acid 49 as a white crystalline solid (0.0078 g, 74%): m.p. 186-188°C. The structure of the product 5 was also confirmed using IR, <sup>1</sup>H NMR and mass spectroscopy.

The preceding examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may be 10 alternatively employed.

Throughout this application various publications are referenced by numbers. Following is a complete citation to the publications. The disclosures of these publications in their 15 entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

## REFERENCES

1. Lotan, R. Biochem. Biophys. Acta 605, 33-91 (1981).
2. Roberts, A.B., & Sporn, M.B. In: The Retinoids (M.B. Sporn, A.B. Roberts, D.S. Goodman, eds). Academic Press, Florida pp. 209-286 (1984).
3. Knudson, D.D. J. Invest. Dermatol. 62,288 (1974).
4. Weiss, J.S., Ellis, C.N., Headington, J.T., Tincoff, T., Hamilton, T.A., & Voorhees, J.J. JAMA 259, 527-532 (1988).
5. Hong, W.K., Lippman, S.M., Itri, L.M., Karp, D.D., Lee, J.S., Byers, R.M., Schantz, S.P., Kramer, A.M., Lotan, R., Peters, L.J., Dimery, I.W., Brown, B.W., & Goepfert, H. N. Engl. J. Med. 323, 795-804 (1990).
6. Huang, M.E., Ye, Y.C., Chen, S., Chai, J.R., Lu, J.X., Lu, L., Zhoa, L., Gu, L.J., & Wang, Z.Y. Blood 72, 567 (1988).
7. Petkovich, M., Brand, N.J., Krust, A., & Chambon, P. Nature 330, 444-450 (1987).
8. Giguere, V., Ong, E.S., Seigi, P., & Evans, R. M. Nature 330, 624-629 (1987).
9. Benbrook, D., Lernhardt, E., & Pfahl, M. Nature 333, 669-672 (1988).
10. Brand, N., Petkovich, M., Krust, A., de Thé, H., Marchio, A., Tiollais, P., & Dejean, A. Nature 332, 850-853 (1988).
11. Krust, A., Kastner, P.H., Petkovich, M., Zelent, A., & Chambon, P. Proc. Nat. Acad. Sci., U.S.A. 86, 5310-5314 (1989).
12. Giguere, V., M. Shago, R. Zirngibl, P., Tate, Rossant, J., & Varmuza, S. Mol. Cell. Biol. 10, 2335-2340 (1990).
13. Hamada, K., Gleason, S.L., Levi, B-Z., Hirschfeld, S., Appella, E., & Ozato, K. Proc. Natl. Acad. Sci., USA 86, 8289-8293 (1989).
14. Mangelsdorf, D. J., Ong, E. S., Dyck, J. A., & Evans, R. M. Nature 345, 224-229 (1990).
15. Yu, V.C., Delsert, C., Andersen, B., Holloway, J.M., Devary, O.V., Näär, A.M., Kim, S.Y., Boutin, J-M., Glass, C.K., & Rosenfeld, M.G. Cell 67, 1251-1266 (1991).
16. Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J-Y., Staub, A., Garnier, J-M., Mader, S., & Chambon, P. Cell 68, 377-395 (1992).
17. Mangelsdorf, D.J., Borgmeyer, U., Heyman, R.A., Zhou, J.Y., Ong, E.S., Oro, A.E., Kakizuka, A., & Evans, R.M. Genes & Dev. 6, 329-344 (1992).

18. Evans, R.M. Science 240, 889-895 (1988).
19. Green, S., & Chambon, P. Trends Genet. 4, 309-314 (1988).
20. Lehmann, J.M., Hoffman, B., & Pfahl, M. Nucl. Acids Res. 19, 573-578 (1991).
21. Leroy, P., Krust, A., Zelent, A., Mendelsohn, C., Garnier, J-M., Kastner, P., Dierich, A., & Chambon, P. EMBO J. 10, 59-69 (1991).
22. Zelent, A., Mendelsohn, C., Kastner, P., Krust, A., Garnier, J-M., Ruffenach, F., Leroy, P., & Chambon, P. EMBO J. 10, 71-81 (1991).
23. Oro, A.E., McKeown, M., & Evans, R.M. Nature 347, 298-301 (1990).
24. Heyman, R., Mangelsdorf, D.J., Dyck, J.A., Stein, R.B., Eichele, G., Evans, R.M., & Thaller, C. Cell 68, 397-406 (1992).
25. Levin, A.A., Sturzenbecker, L.J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzeisen, C., Rosenberger, M., Lovey, A., & Grippo, J.F. Nature 355, 359-361 (1992).
26. Zhang, X-k., Hoffmann, B., Tran, P., Graupner, G., & Pfahl, M. Nature 355, 441-446 (1992).
27. Kliewer, S.A., Umesono, K., Mangelsdorf, D.J., & Evans, R.M. Nature 355, 446-449 (1992).
28. Marks, M.S., Hallenbeck, P.L., Nagata, T., Segars, J.H., Apella, E., Nikodem, V.M., & Ozato, K. EMBO J. 11, 1419-1435 (1992).
29. Bugge, T.H., Pohl, J., Lonnay, O., & Stunnenberg, H.G. EMBO J. 11, 1409-1418 (1992).
30. Mangelsdorf, D.J., Umesono, K., Kliewer, S.A., Borgmeyer, U., Ong, E.S., & Evans, R.M. Cell 66, 555-561 (1991).
31. Rottman, J.N., Widom, R.L., Nadal-Ginard, B., Mahdavi, V., & Karathanasis, S.K. Mol. Cell. Biol. 11, 3814-3820 (1991).
32. Hermann, T., Hoffmann, B., Zhang, X-k., Tran, P. & Pfahl, M. Mol. Endocrinol. (accepted for publication).
33. Zhang, X-k., Tran, P., & Pfahl, M. Mol. Endocrinol. 5, 1909-1920 (1991b).
34. Glass, C.K., Holloway, J.M., Devary, O.V., & Rosenfeld, M.G. Cell 54, 313-323 (1988).
35. de Thé, H., Vivanco-Ruiz, M.M., Tiollais, P., Stunnenberg, H., & Dejean, A. Nature 343, 177-180 (1990).
36. Hoffmann, B., Lehmann, J.M., Zhang, X-k., Hermann, T., Graupner, G., & Pfahl, M. Mol. Endocrinol. 4, 1734-1743 (1990).
37. Husmann, M.B., Hoffmann, B., Stump, D.G., Chyttil, F., & Pfahl, M.

BBRC, in press (1992).

39. Umesono, K., Murakami, K.K., Thompson, C.C., & Evans, R.M. Cell 65, 1255-1266 (1991).
40. Flink, I.L., & Morkin, E. J. Biol. Chem. 265, 11233-11237 (1990).
41. Desvergne, B., Petty, K.J., & Nikodem, V.M. J. Biol. Chem. 266, 1008-1013 (1991).
42. Klein-Hitpass, L., Schorpp, M., Wagner, U., & Ryffel, G.U. Cell 46, 1053-1061 (1986).
43. Husmann, M., Lehmann, J., Hoffmann, B., Hermann, T., Tzukerman, M., & Pfahl, M. Mol. Cell. Biol. 11, 4097-4103 (1991).
44. Tora, L., White, J., Brou, C., Tasset, D., Webster, N., Scheer, E., & Chambon, P. Cell 59, 477-487 (1989).
45. Tzukerman, M., Zhang, X-k., Wills, K.N., Graupner, G., Hermann, T., & Pfahl, M. New Biol. 2, 613-620 (1990).
46. Zhang, X-k., Wills, K.N., Graupner, G., Tzukerman, M., Hermann, T., & Pfahl, M. New Biol. 3, 1-14 (1991a).
47. Yen, P.M., Darling, D.S., Carter, R.L., Forgione, M., Umeda, P.K., & Chin, W.W. J. Biol. Chem. 267, 3565-3568 (1992).
48. Forman, B.M., & Samuels, H.H. Mol. Endocrinol. 4, 1293-1301 (1990).
49. Zenke, M., Munoz, A., Sap, J., Vennstrom, B., & Beug, H. Cell 61, 1035-1049 (1990).
50. Yang-Yen, H-F., Zhang, X-k., Graupner, G., Tzukerman, M., Sakamoto, B., Karin, M., & Pfahl, M. New Biol. 3, 1206-1219 (1991).
51. X-k., Zhang, B., Hoffmann, P., Tran, G., Graupner, M., Pfahl, Nature 355, 441 (1992).
52. Pfahl et al., Methods Enzymol. 153, 256 (1990).

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (A) NAME: The Government of the United States of America, as represented by The Secretary
- (B) STREET: 6011 Executive Blvd., Suite 325
- (C) CITY: Rockville
- (D) STATE: Maryland
- (E) COUNTRY: United States of America
- (F) POSTAL CODE (ZIP): 20852
- (G) TELEPHONE: (301) 496-7056
- (H) TELEFAX: (301) 402-0220
- (I) TELEX: None

(ii) TITLE OF INVENTION: RXR HOMODIMER FORMATION

(iii) NUMBER OF SEQUENCES: 11

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(v) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: NOT YET ASSIGNED
- (B) FILING DATE:

(vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US/07/901/719
- (B) FILING DATE: 16-JUN-1992

## (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Arg Tyr Lys Asp Asp Asp Asp Lys  
1 5

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GATCAGGGCA GGGGTCAAGG GTTCAGTGAT C

31

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GATCCAGGTC ACAGGTACA GGTACACAGTT CAAGATC

37

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GATCTGTAGG GTTCACCGAA AGTTCACTCA GATC

34

## (2) INFORMATION FOR SEQ ID NO:5:

## 3 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

70

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATCCAGGTC AAAAAGTCAG GATC

24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GATCCTGGAG GTGACAGGAG GACAGCGATC

30

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GATCCAGGAC GTTGGGGTTA GGGGGAGGACA GTGGGATC

38

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GATCTCAGGT CATCCTCAGG TCAGATC

27

71

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GATCTCAGGT CATCCTCAGG TCAGATC

27

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GATCTCAGGT CACTGTGACC TGAGATC

27

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GATCTCAGGT CATGACCTGA GATC

24

What is claimed is:

1. A method of screening a substance for the ability to affect the formation of a retinoid X receptor homodimer comprising combining the substance and a solution containing retinoid X receptors and determining the presence of homodimer formation.
2. The method of claim 1, wherein the presence of homodimer formation is determined by detecting the activation of transcription by the retinoid X receptor homodimer.
3. The method of claim 1, wherein the presence of homodimer formation is determined by coprecipitation.
4. The method of claim 1, wherein the effect is the induction of homodimer formation.
5. The method of claim 1, wherein the effect is the inhibition of homodimer formation.
6. The method of claim 1, wherein the retinoid X receptor is retinoid X receptor  $\alpha$ .
7. The method of claim 4, further comprising determining if the substance induces a retinoid X receptor heterodimer and selecting the substance which selectively induces the formation of a retinoid X receptor homodimer.
8. A method of screening a substance for the ability to selectively induce the formation of a retinoid X receptor heterodimer over a retinoid X receptor homodimer comprising:
  - a) determining if the substance induces retinoid X receptor homodimer formation;
  - b) determining if the substance induces retinoid X receptor heterodimer formation; and
  - c) selecting the compound which selectively induces the formation of a retinoid X receptor heterodimer over a retinoid X receptor homodimer.

homodimer.

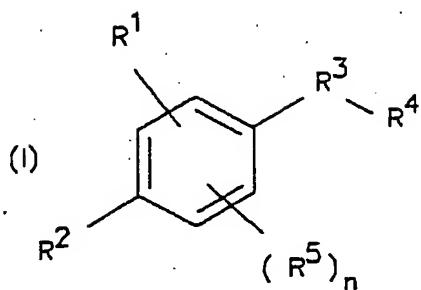
9. A method of screening a substance for an affect on a retinoid X receptor homodimer's ability to bind DNA comprising combining the substance with the homodimer and determining the affect of the compound on the homodimer's ability to bind DNA.

10. A method of screening a response element for binding with a retinoid X receptor homodimer comprising combining the response element with the retinoid X receptor homodimer and detecting the presence of binding.

11. The method of claim 10, wherein the presence of binding is detected by the transcriptional activation of a marker which is operably linked to the response element.

12. A purified retinoid X receptor homodimer.

## 13. A bicyclic aromatic compound having the structural formula

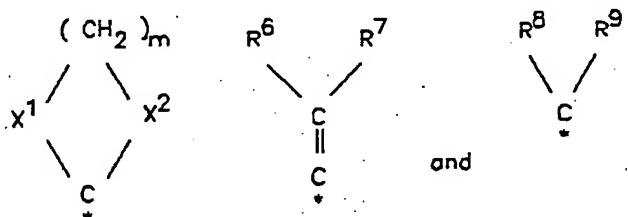


wherein:

R¹ is selected from the group consisting of lower alkyl and adamantlyl;

R² is -O-R⁶ or -S-R⁶ where R⁶ is lower alkyl; or where R¹ is ortho to R², R¹ and R² may be linked together to form a 5- or 6-membered cycloalkylene ring, either unsubstituted or substituted with 1 to 4 lower alkyl groups, and optionally containing 1 or 2 heterocyclic atoms selected from the group consisting of O, S and NR where R is hydrogen or lower alkyl;

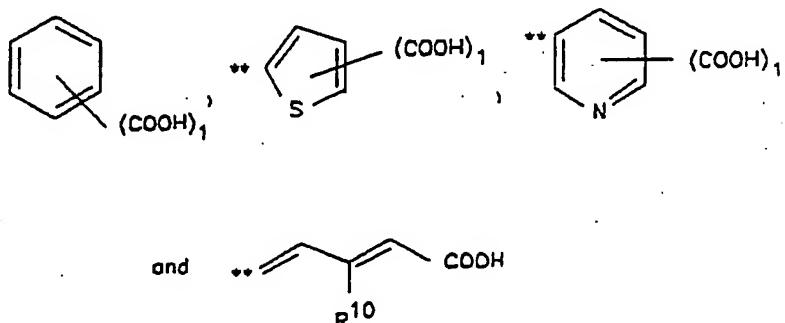
R³ is selected from the group consisting of carbonyl,



in which X¹ and X² are independently selected from the group consisting of O, S and methylene, wherein at least one of X¹ and X² is O or S, or wherein one of X¹ and X² is NR and the other is methylene, m is 2 or 3, R⁶, R⁷, R⁸ and R⁹ are independently hydrogen or lower alkyl, with the proviso that when n is 0, R⁶ and R⁷ are not both hydrogen and R⁸ and R⁹

are not both hydrogen, or R<sup>8</sup> and R<sup>9</sup> may be linked together to form a cycloalkylene ring containing 3 to 6 carbon atoms, and \* represents the point of attachment of the R<sup>3</sup> substituent to the remainder of the molecule; and

R<sup>4</sup> is selected from the group consisting of



in which R<sup>10</sup> is hydrogen or methyl, 1 is 0 or 1, and \*\* represents the point of attachment of the R<sup>4</sup> substituent to the remainder of the molecule,

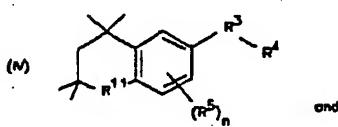
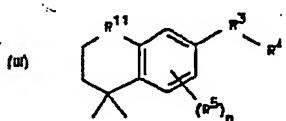
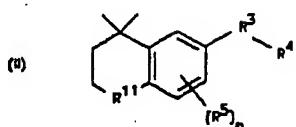
the R<sup>5</sup> are independently selected from the group consisting of lower alkyl and lower alkoxy; and

n is 0, 1, 2 or 3,

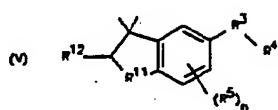
with the proviso that if n is 0, R<sup>3</sup> is other than carbonyl, \*C=CH<sub>2</sub> or CH<sub>2</sub>,

and pharmaceutically acceptable esters, amides and salts of the compound.

14. The compound of claim 13, having a structure selected from the group consisting of



and

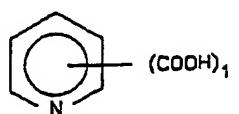


where R<sup>11</sup> is selected from the group consisting of O, S, (CH<sub>3</sub>)<sub>2</sub>C and CH<sub>2</sub>, and R<sup>12</sup> is hydrogen or methyl.

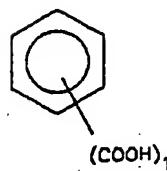
15. The compound of claim 14, having the structural formula (II).

16. The compound of claim 15, wherein n is 0.

17. The compound of claim 16, wherein R<sup>4</sup> is

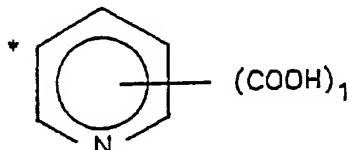


or

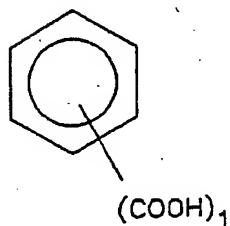


and 1 is 1.

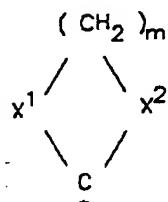
18. The compound of claim 17, wherein R<sup>4</sup> is



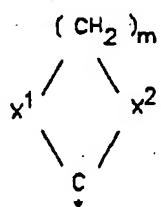
19. The compound of claim 17, wherein R<sup>4</sup> is



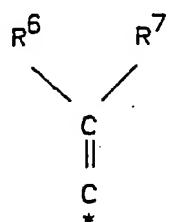
20. The compound of claim 15, wherein R<sup>3</sup> has the structural formula



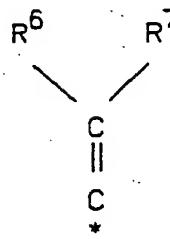
21. The compound of claim 17, wherein R<sup>3</sup> has the structural formula



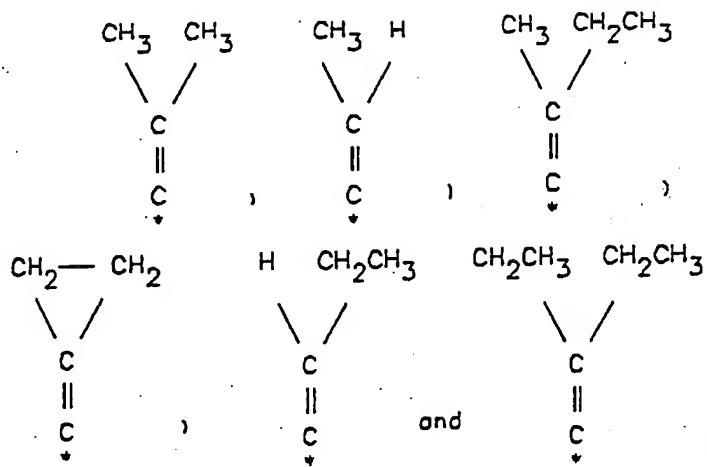
22. The compound of claim 15, wherein R<sup>3</sup> has the structural formula



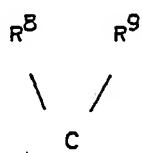
23. The compound of claim 17, wherein R<sup>3</sup> has the structural formula



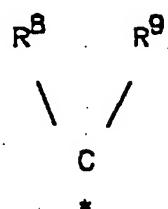
24. The compound of claim 23, wherein R<sup>3</sup> is selected from the group consisting of



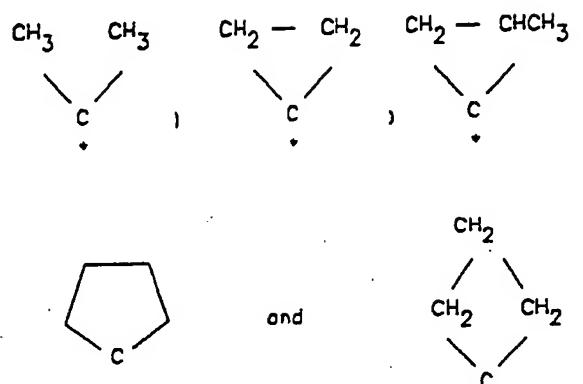
25. The compound of claim 15, wherein R<sup>3</sup> has the structural formula



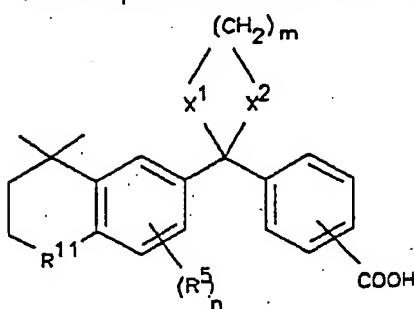
26. The compound of claim 17, wherein R<sup>3</sup> has the structural formula



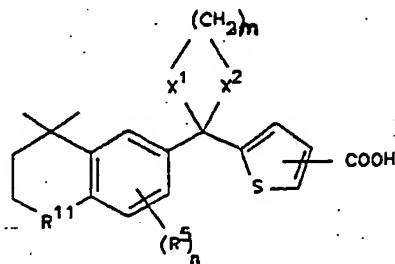
27. The compound of claim 26, wherein R<sup>3</sup> is selected from the group consisting of



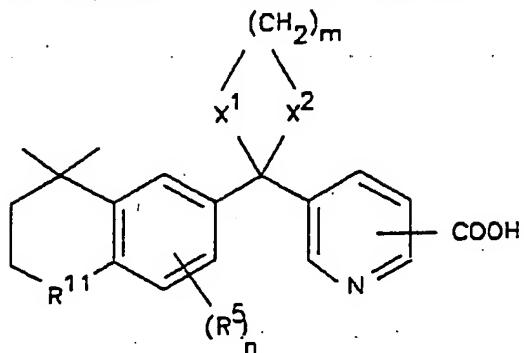
28. The compound of claim 15, having the structural formula



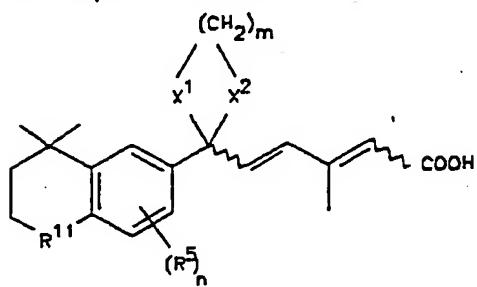
29. The compound of claim 15, having the structural formula



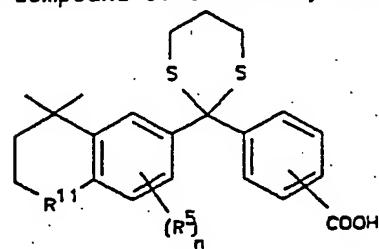
30. The compound of claim 15, having the structural formula



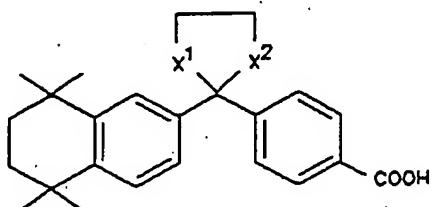
31. The compound of claim 15, having the structural formula



32. The compound of claim 15, having the structural formula

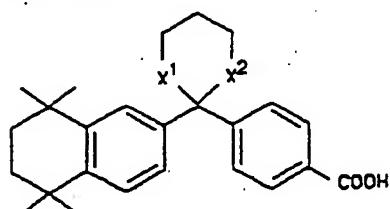


33. The compound of claim 15, having the structural formula



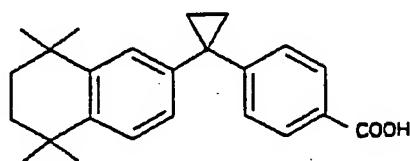
wherein X<sup>1</sup> and X<sup>2</sup> are independently selected from the group consisting of O and S, and the ammonium salt of the compound.

34. The compound of claim 15, having the structural formula

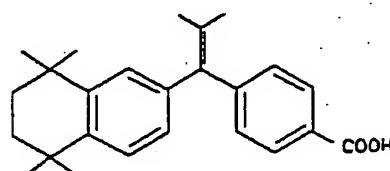


wherein X<sup>1</sup> and X<sup>2</sup> are independently selected from the group consisting of O and S, and the ammonium salt of the compound.

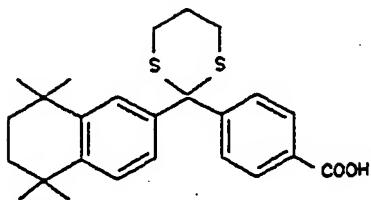
35. The compound of claim 15, having the structural formula



36. The compound of claim 15, having the structural formula

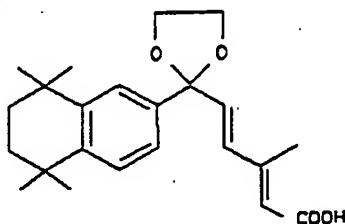


37. The compound of claim 15, having the structural formula



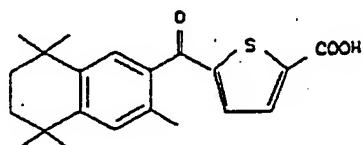
wherein R<sup>1</sup> is O or S.

38. The compound of claim 15, having the structural formula

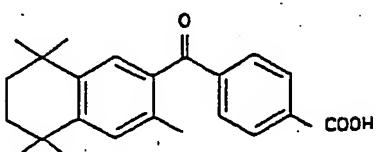


and the ammonium salt of the compound.

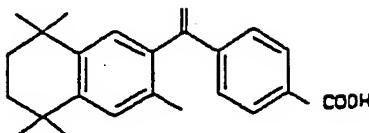
39. The compound of claim 15, having the structural formula



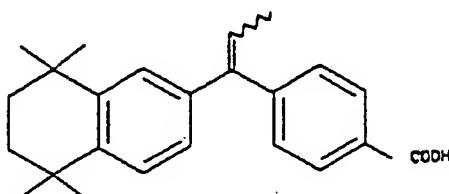
40. The compound of claim 15, having the structural formula



41. The compound of claim 15, having the structural formula



42. The compound of claim 15, having the structural formula



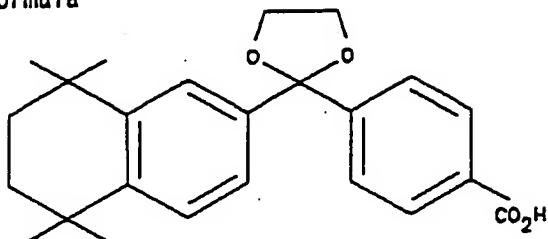
43. A method of inhibiting an activity of a retinoid X receptor heterodimer comprising inducing the formation of a retinoid X receptor homodimer, thereby preventing the retinoid X receptor from forming a heterodimer and preventing the resulting heterodimer activity.

44. The method of claim 43, wherein the activity is the activation or repression of transcription.

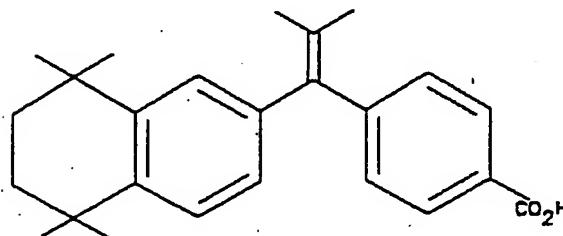
45. The method of claim 43, wherein the retinoid X receptor heterodimer is thyroid hormone receptor and retinoid X receptor.

46. A method of promoting the transcription of a gene activated by a retinoid X receptor homodimer in a cell comprising contacting the cell with an amount of a synthetic compound capable of promoting the formation of retinoid X receptor homodimers.

47. The method of claim 46, wherein the compound has the structural formula



48. The method of claim 46, wherein the compound has the structural formula



49. A method of inhibiting an activity of a retinoid X receptor homodimer comprising preventing the formation of the retinoid X receptor homodimer.

50. The activity of claim 49, wherein the activity is the activation or repression of transcription.

51. A method of inhibiting an activity of a retinoid X receptor homodimer comprising preventing the binding of the retinoid X receptor homodimer to its response element.

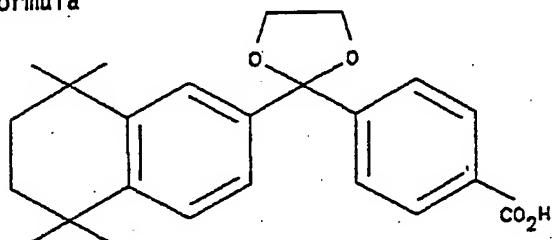
52. The method of claim 51, wherein the activity is the activation or repression of transcription.

53. A method of determining an increased probability of a pathology associated with retinoid X receptor homodimer formation comprising

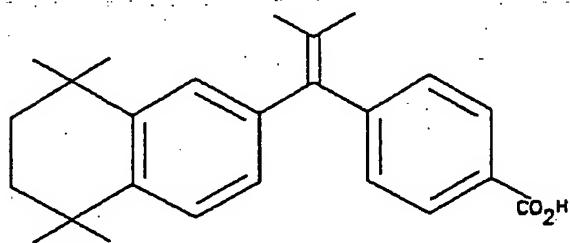
detecting a decrease of retinoid X receptor homodimer formation in the subject when compared to a normal subject.

54. A method of treating a pathology associated with retinoid X receptor homodimers in a subject comprising inducing retinoid X receptor homodimer formation in the subject, thereby increasing the expression of a gene which can be activated by a retinoid X receptor homodimer.

55. The method of claim 54, wherein retinoid X receptor homodimer formation is induced by the addition of a compound having the structural formula



56. The method of claim 54, wherein retinoid X receptor homodimer formation is induced by the addition of a compound having the structural formula



57. The method of claim 54, wherein the pathology is associated with the skin.

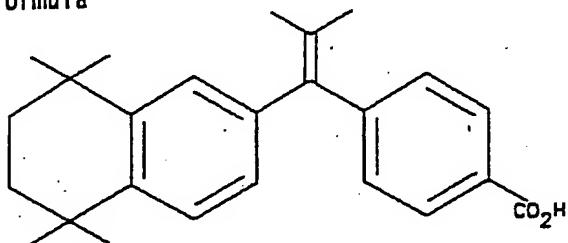
58. The method of claim 54, wherein the skin pathology is selected from the group consisting of acne and psoriasis.

59. The method of claim 54, wherein the pathology is a cancer.

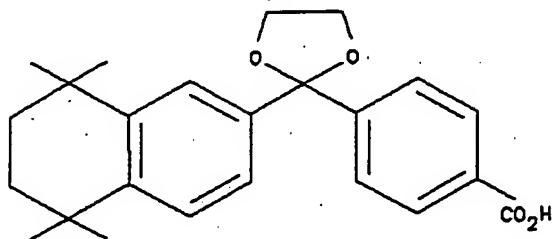
60. The method of claim 54, wherein the gene is the apolipoprotein AI gene.

61. A method of selectively activating retinoid X receptor homodimer formation in a cell comprising adding to the cell a homodimer formation specific ligand, thereby selectively activating the retinoid X receptor homodimer formation in the cell.

62. The method of claim 61, wherein the compound has the structural formula



63. The method of claim 61, wherein the compound has the structural formula



64. A method of promoting retinoid X receptor homodimer formation in a cell comprising the expression of 9-cis-RA in the cell.

65. A pharmaceutical composition for control of cellular processes regulated by retinoic acid, vitamin D, or thyroid hormone, comprising an

effective regulating amount of the compound of claim 13 in combination with a pharmaceutically acceptable carrier.

66. A pharmaceutical composition for control of cellular processes regulated by retinoic acid, vitamin D, or thyroid hormone, comprising an effective regulating amount of the compound of claim 15 in combination with a pharmaceutically acceptable carrier.

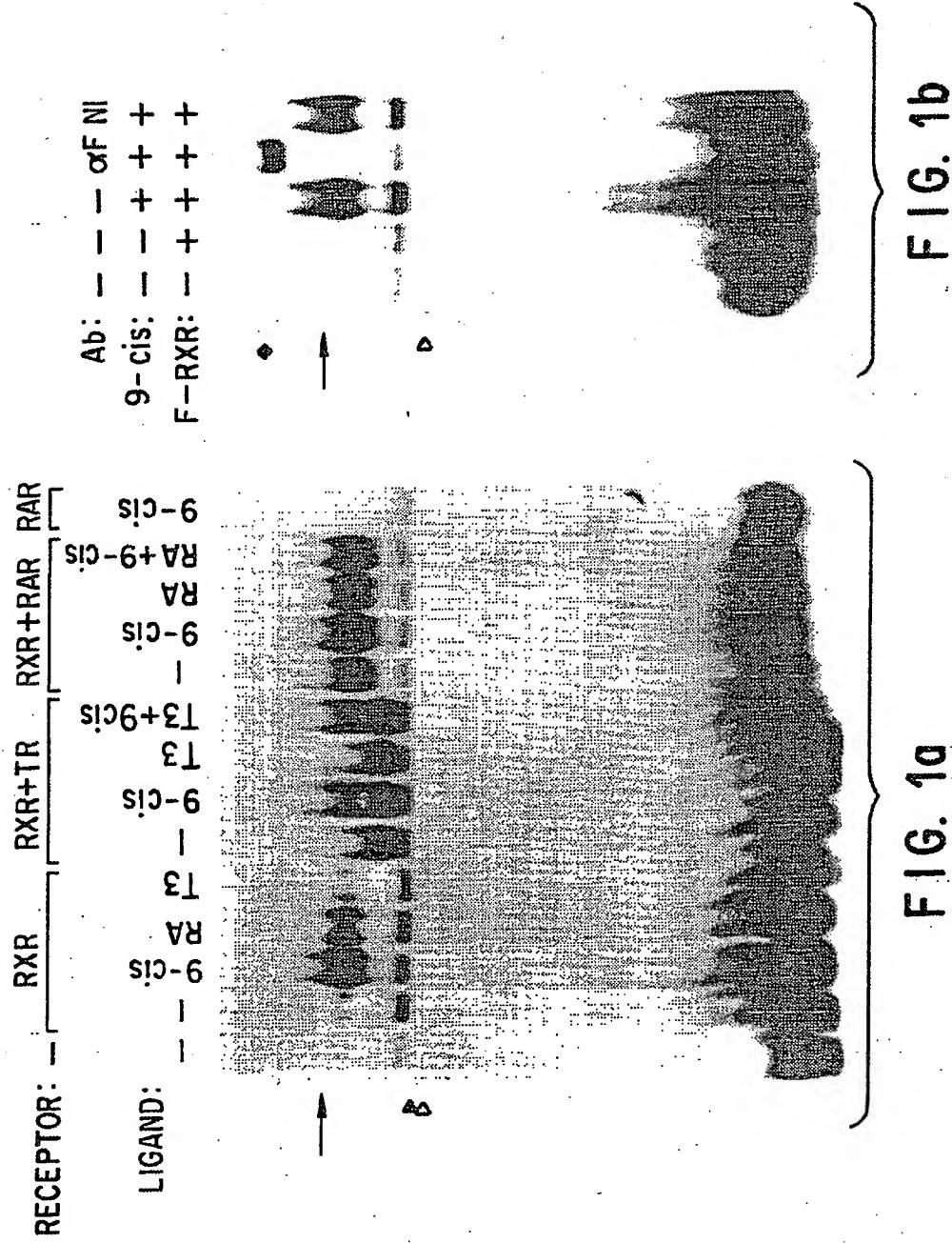
67. A method for modulating gene expression, in a subject, by a receptor selected from the group consisting of retinoic acid receptors, retinoid X receptors, vitamin D receptors and thyroid receptors, comprising administering to the subject an effective modulating amount of the compound of claim 13 or a pharmaceutical composition thereof.

68. A method for modulating gene expression, in a subject, by a receptor selected from the group consisting of retinoic acid receptors, retinoid X receptors, vitamin D receptors and thyroid receptors, comprising administering to the subject an effective modulating amount of the compound of claim 15 or a pharmaceutical composition thereof.

69. A method for treating a subject afflicted with a disease caused by malfunction of cell differentiation processes regulated by retinoids, thyroid hormone, or vitamin D, comprising administering to the subject a therapeutically effective amount of the compound of claim 13 or a pharmaceutical composition thereof.

70. A method for treating a subject afflicted with a disease caused by malfunction of cell differentiation processes regulated by retinoids, thyroid hormone, or vitamin D, comprising administering to the subject a therapeutically effective amount of the compound of claim 15 or a pharmaceutical composition thereof.

1/13

RECTIFIED SHEET (RULE 91)  
ISA/EP

2/13

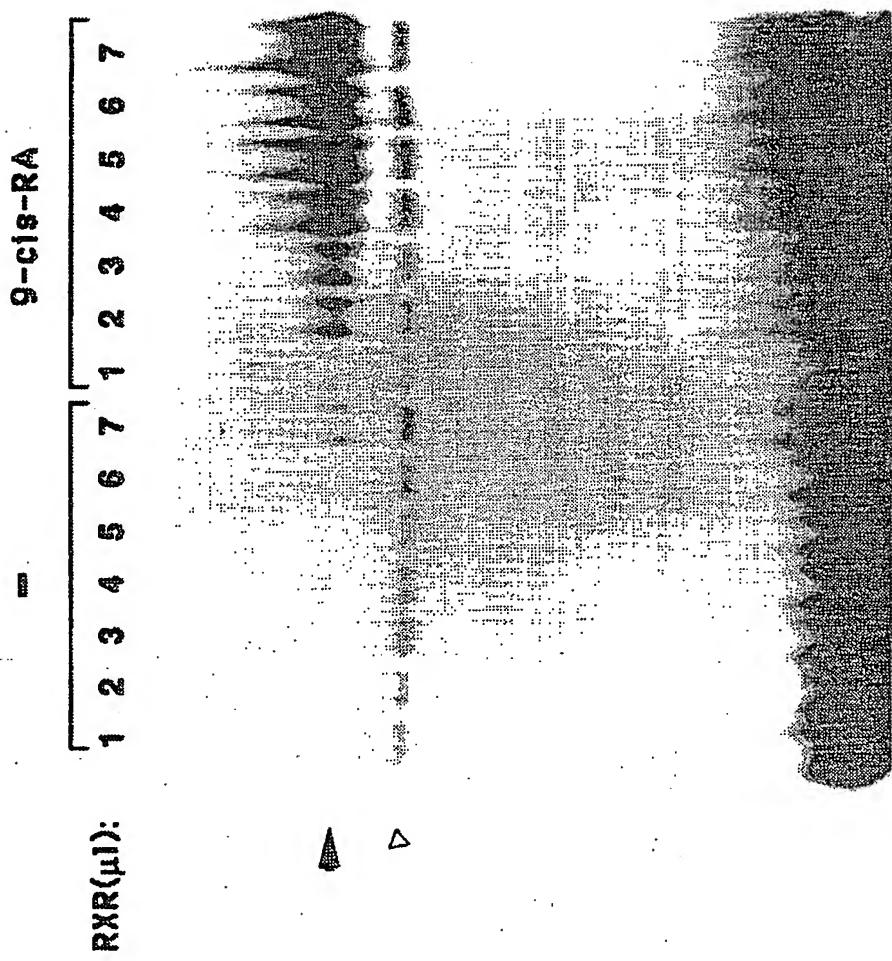


FIG. 20

RECTIFIED SHEET (RULE 91)  
ISA/EP

3/13

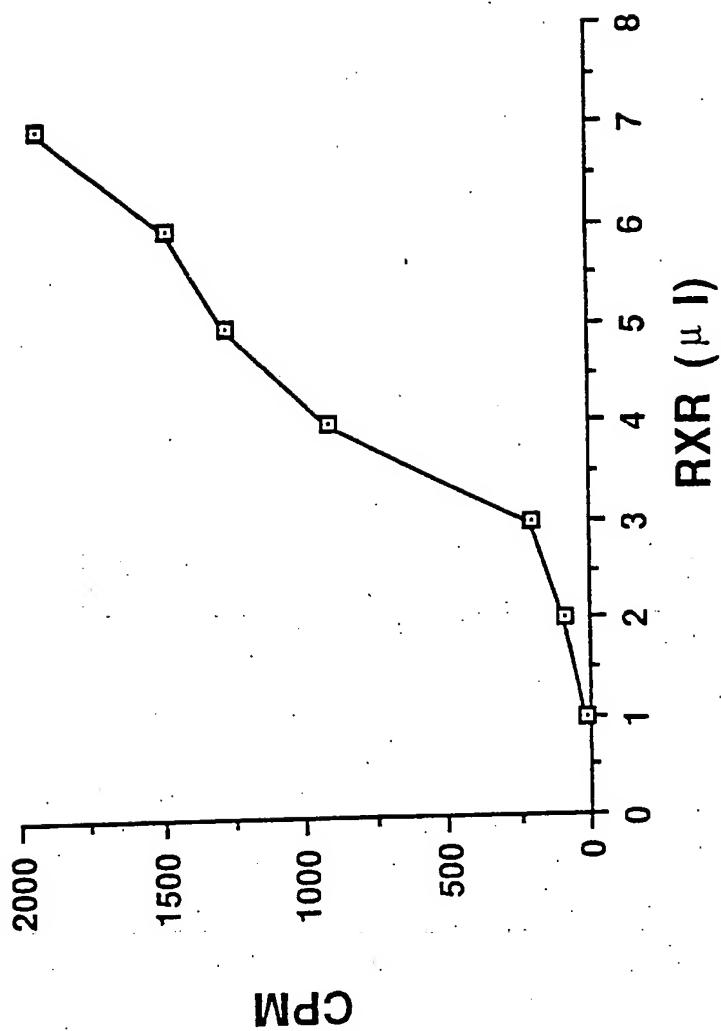


FIG. 2b

RECTIFIED SHEET (RULE 91)  
ISA/EP

4/13

$-\log[9\text{-cis-RA}][\text{M}]$ : - 10 9 8 7 6 5

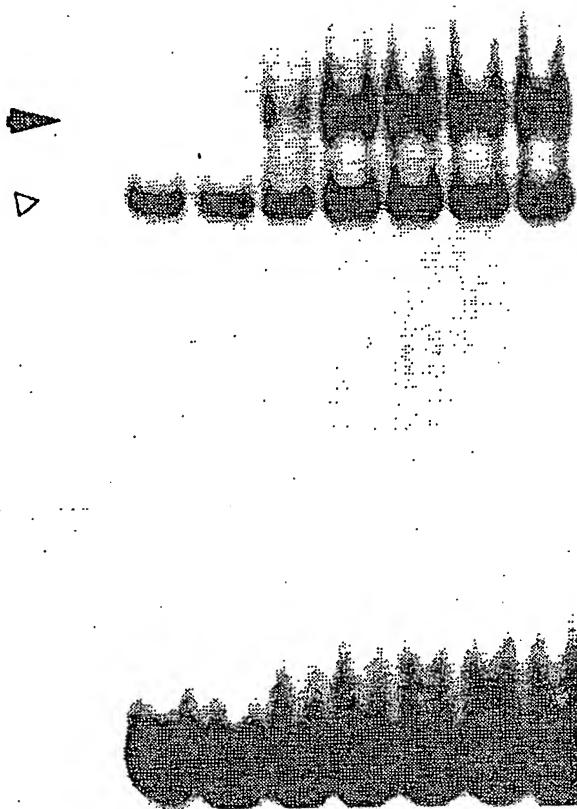


FIG. 2c

RECTIFIED SHEET (RULE 91)  
ISA/EP

5/13

|              |   |
|--------------|---|
| TREpal       | gatcTCAGGTCA <u>TGACCTG</u> Agatc<br>ctag <u>AGTCCAGTACTGGACT</u> ctag  |
| ApoAI-RARE   | gatcAGGGCAGGGGTCA <u>AGGGTT</u> CAGTgatc<br>ctag <u>TCCCAGTCCCCAGT</u> <u>TC</u> <u>CAAGT</u> CAActag   |
| CRBPII-RARE  | gatcCAGGT <u>CACAGGT</u> CACAGGT <u>CACAGT</u> TCAAgatc<br>ctag <u>GTCCAGT</u> <u>GTCCAGT</u> <u>GTCCAGT</u> <u>GTCAAGT</u> TTctag  |
| $\beta$ RARE | gatctGTAGGGTT <u>CACCGAA</u> AGTT <u>CACT</u> Ca <u>gatc</u><br>ctaga <u>CAT</u> <u>CCCAAGT</u> <u>GGCTT</u> <u>CAAGT</u> <u>GAGt</u> ctag  |
| CRBPI-RARE   | gatccAGGT <u>AAAAAAGT</u> CAG <u>gatc</u><br>ctagg <u>TCCAGT</u> <u>TTTCAGT</u> <u>C</u> ctag   |
| MHC-TRE      | gatc <u>CTGGAGGT</u> GAC <u>AGGGAGG</u> A <u>AGC</u> gatc<br>ctag <u>GACCTCC</u> <u>ACTG</u> <u>TCCT</u> <u>GT</u> <u>CG</u> ctag   |
| ME-TRE       | gatc <u>CAGGACGT</u> <u>GGGTT</u> <u>AGGGAGG</u> A <u>AGTGG</u> gatc<br>ctag <u>GTCCTG</u> <u>CAACCC</u> <u>CA</u> <u>AT</u> <u>CCCC</u> <u>CCTG</u> <u>TG</u> <u>CA</u> <u>CC</u> ctag |
| DR-4         | gatcTCAGGT <u>CATCT</u> <u>CAGGT</u> CA <u>gatc</u><br>ctag <u>AGTCC</u> <u>AGT</u> <u>AGT</u> <u>CCAGT</u> ctag  |
| DR-5         | gatcTCAGGT <u>CATC</u> <u>C</u> <u>TCAGGT</u> CA <u>gatc</u><br>ctag <u>AGTCC</u> <u>AGT</u> <u>AGGAGT</u> <u>CCAGT</u> ctag  |
| ERE          | gatcTCAGGT <u>CACT</u> <u>GT</u> <u>GACCT</u> <u>G</u> Agatc<br>ctag <u>AGTCC</u> <u>AGT</u> <u>GAC</u> <u>ACT</u> <u>GG</u> <u>ACT</u> ctag  |

FIG. 3a  
RECTIFIED SHEET (RULE 91)  
ISA/EP

6/13

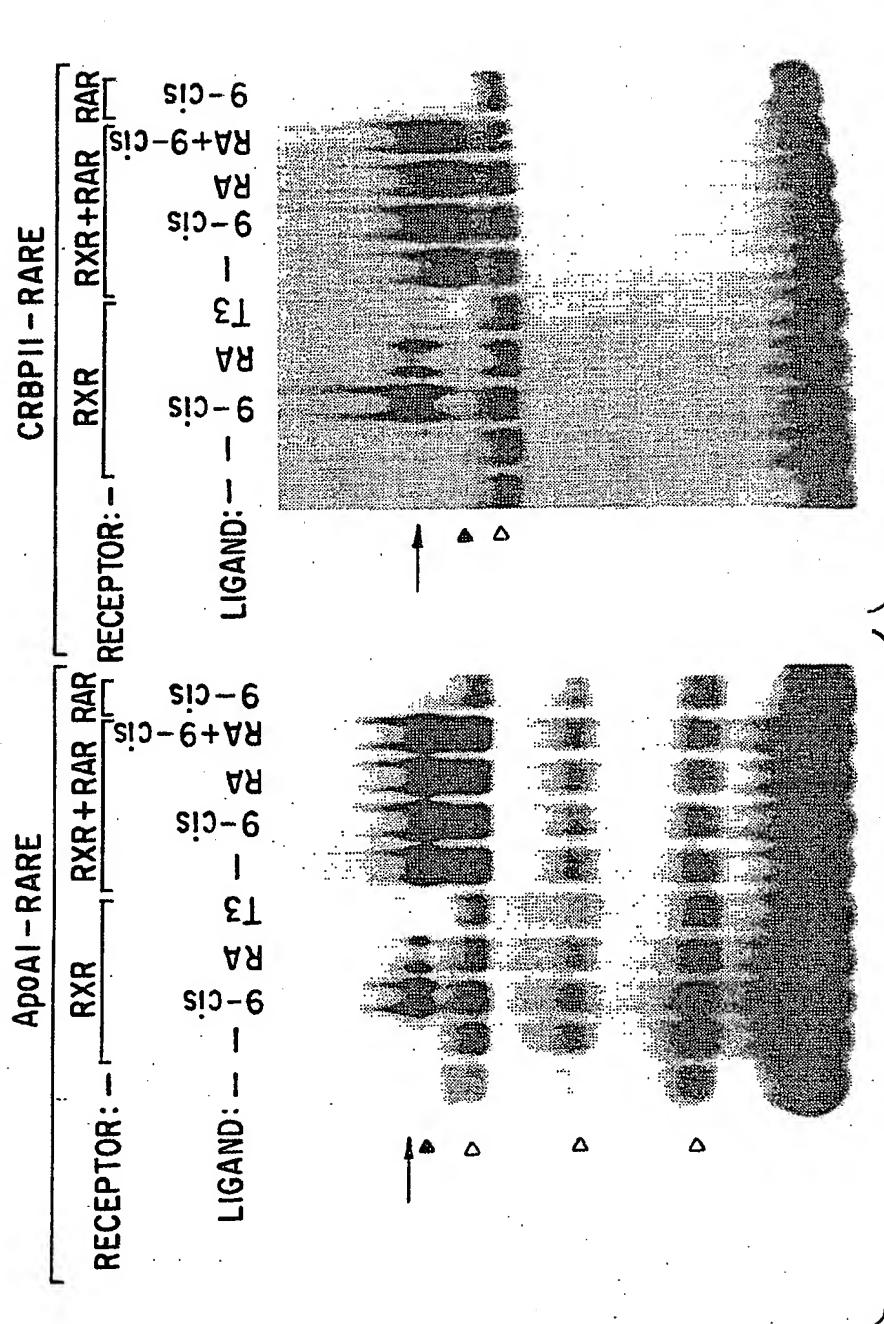
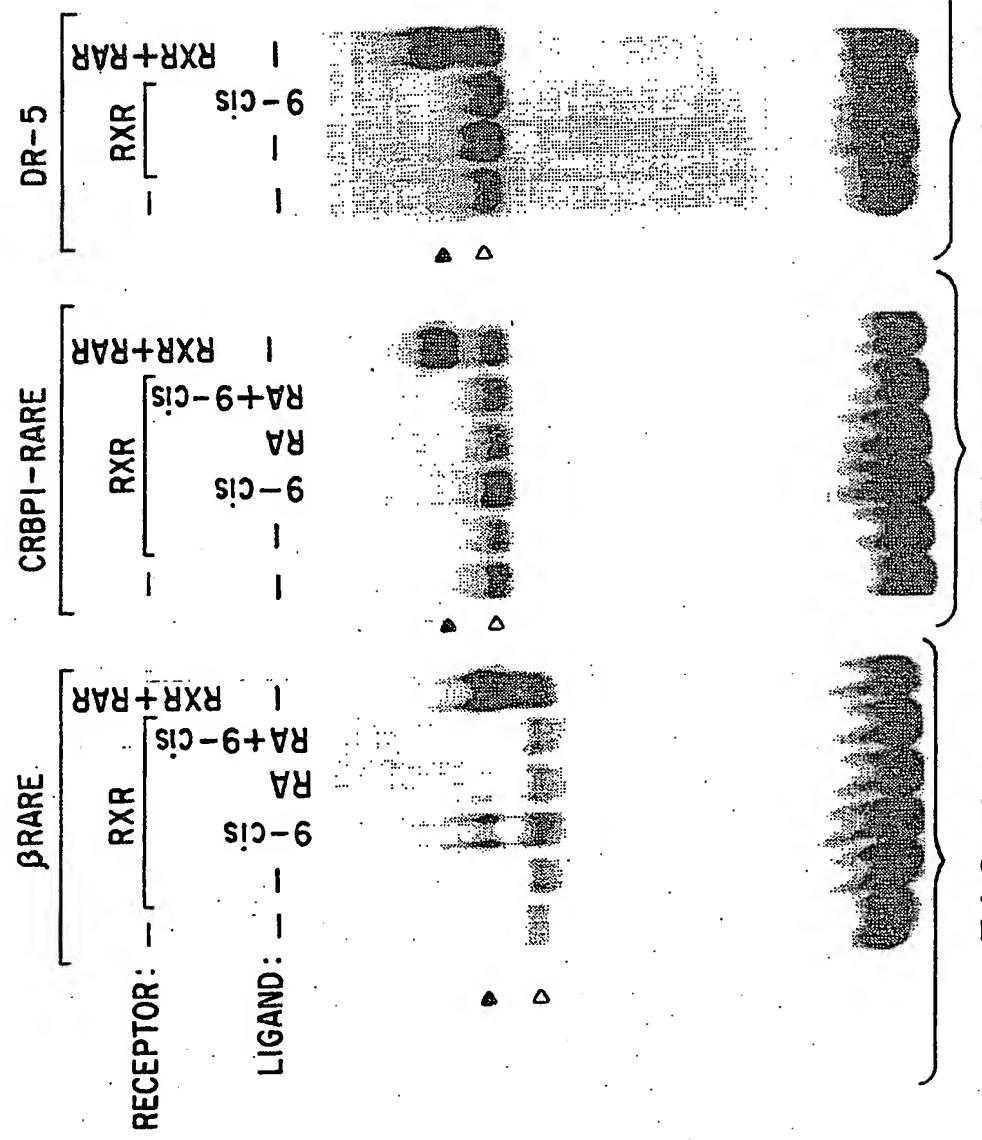


FIG. 3b

FIG. 3c

RECTIFIED SHEET (RULE 91)  
ISA/EP

7/13



8/13

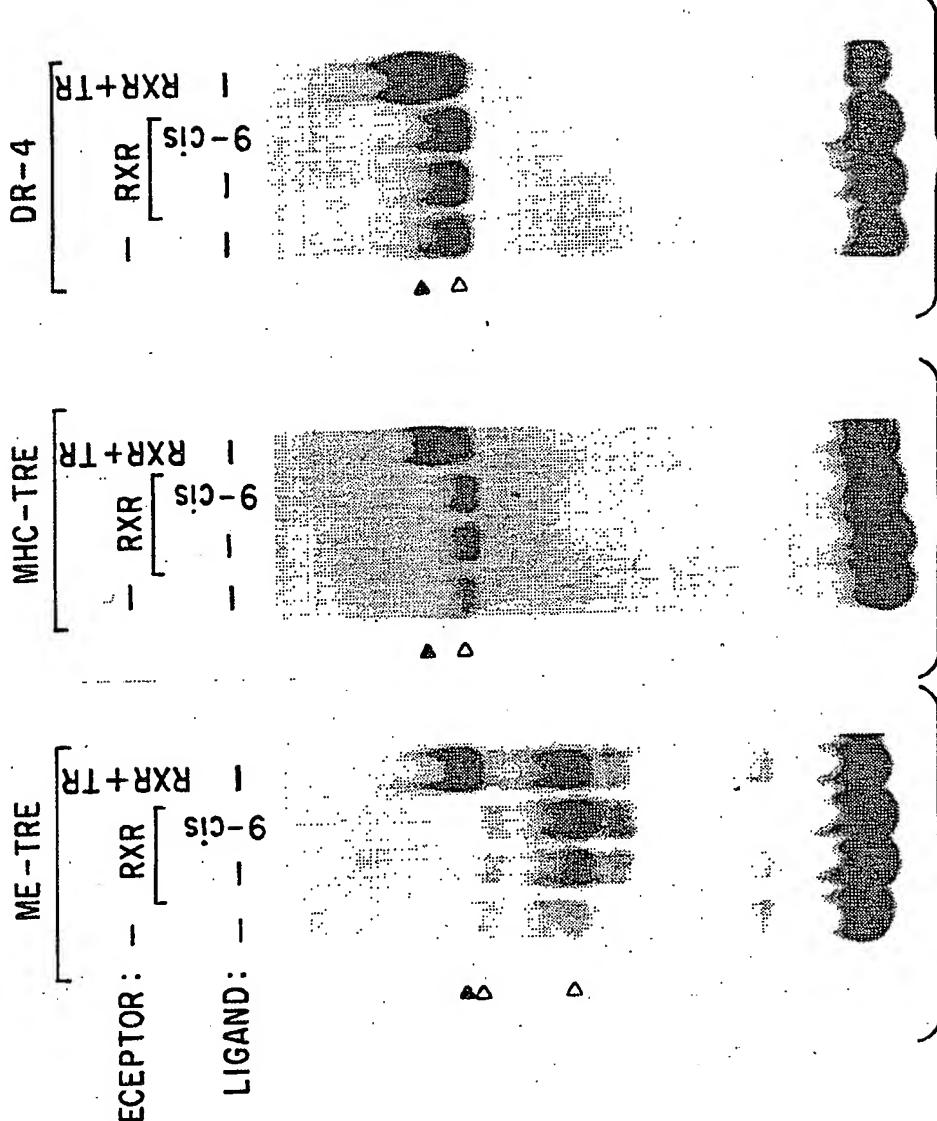


FIG. 4f

FIG. 4e

FIG. 4d

RECTIFIED SHEET (RULE 91)  
ISA/EP

9/13

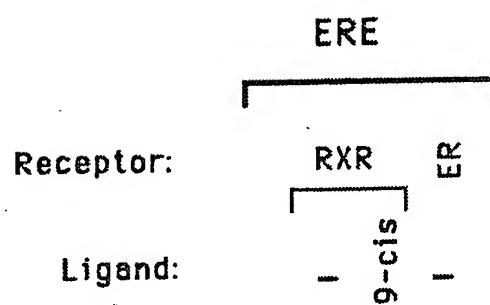
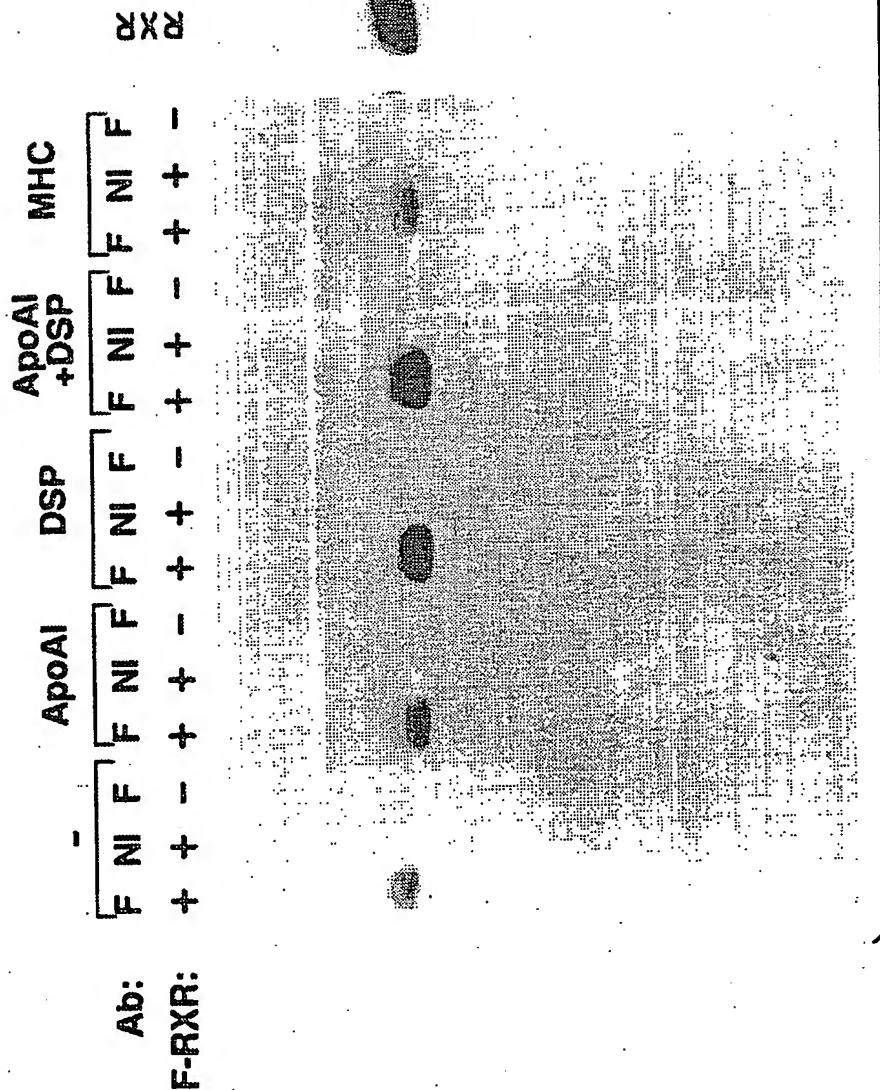


FIG. 4g

10/13



RECTIFIED SHEET (RULE 91)  
ISA/EP

11/13

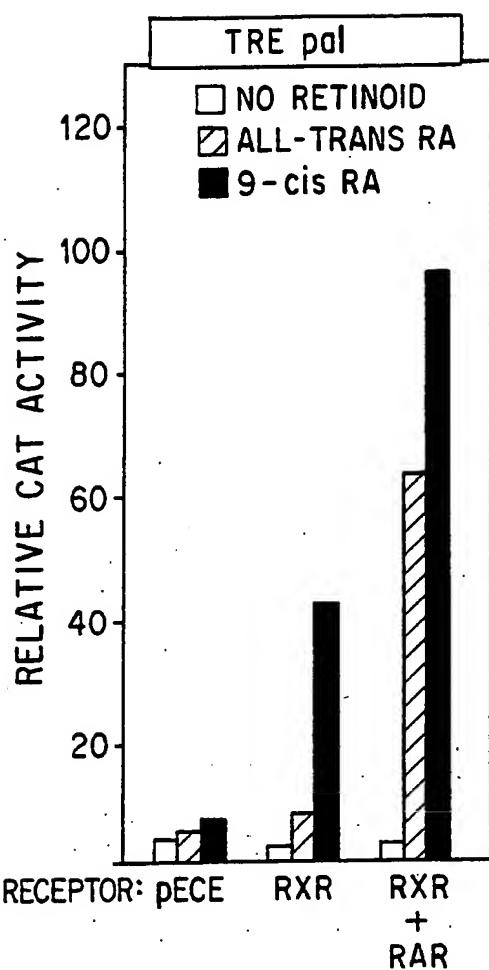


FIG. 6a

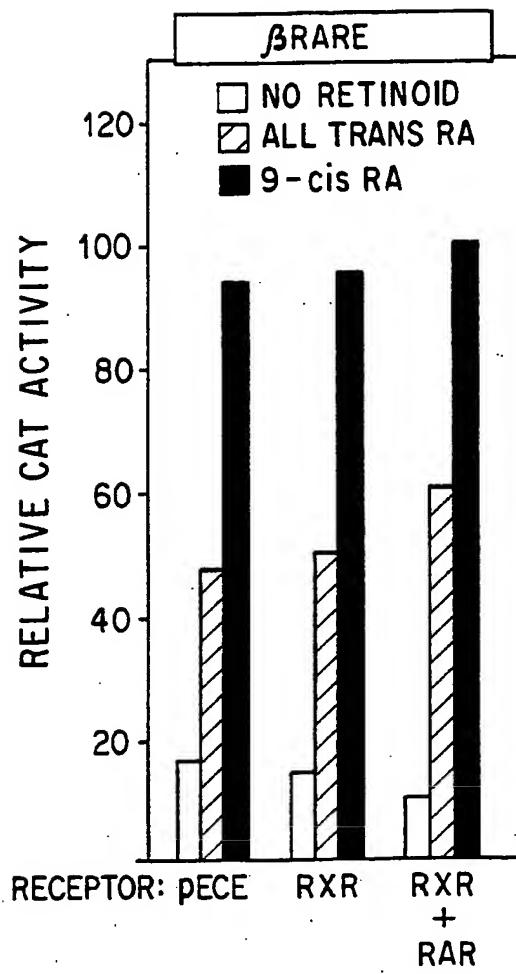


FIG. 6b

12/13

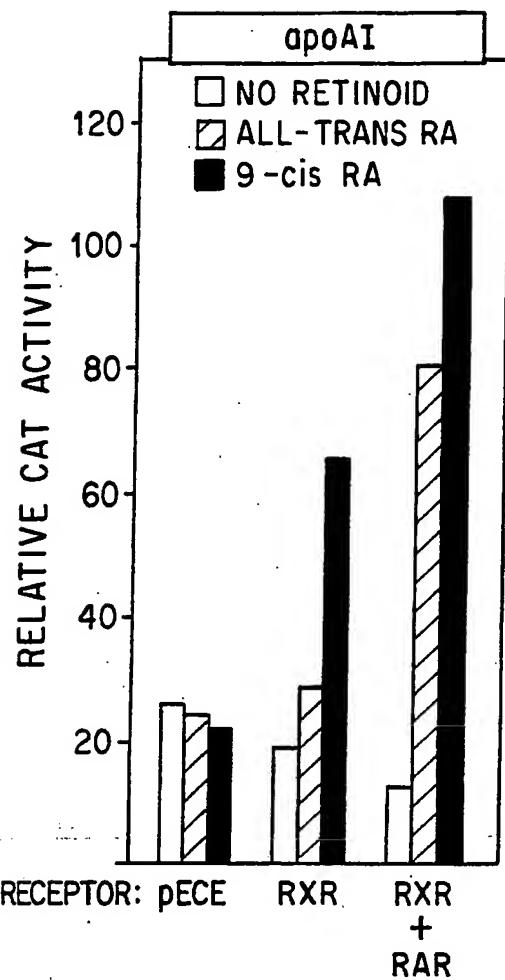


FIG. 6c

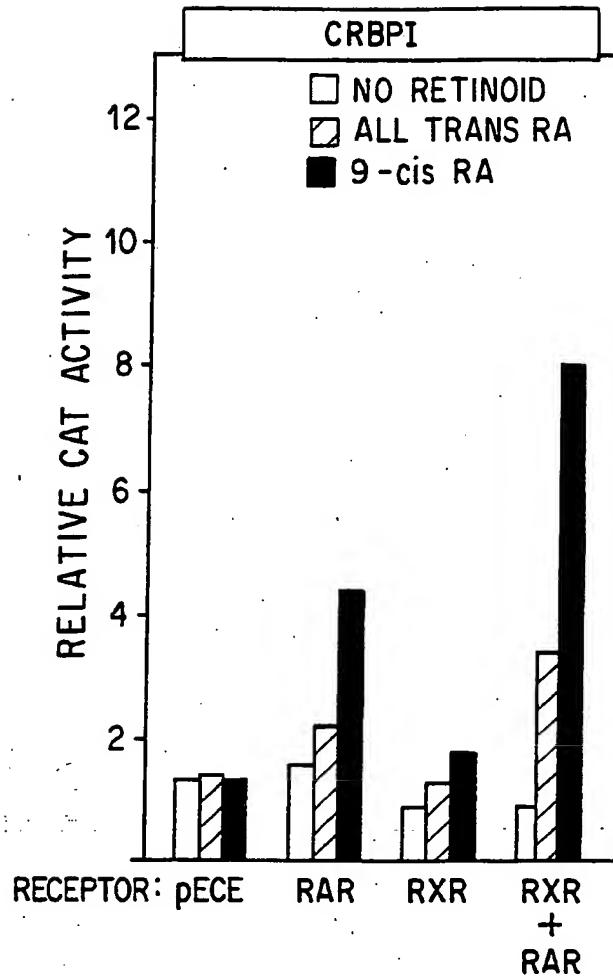


FIG. 6d

13/13

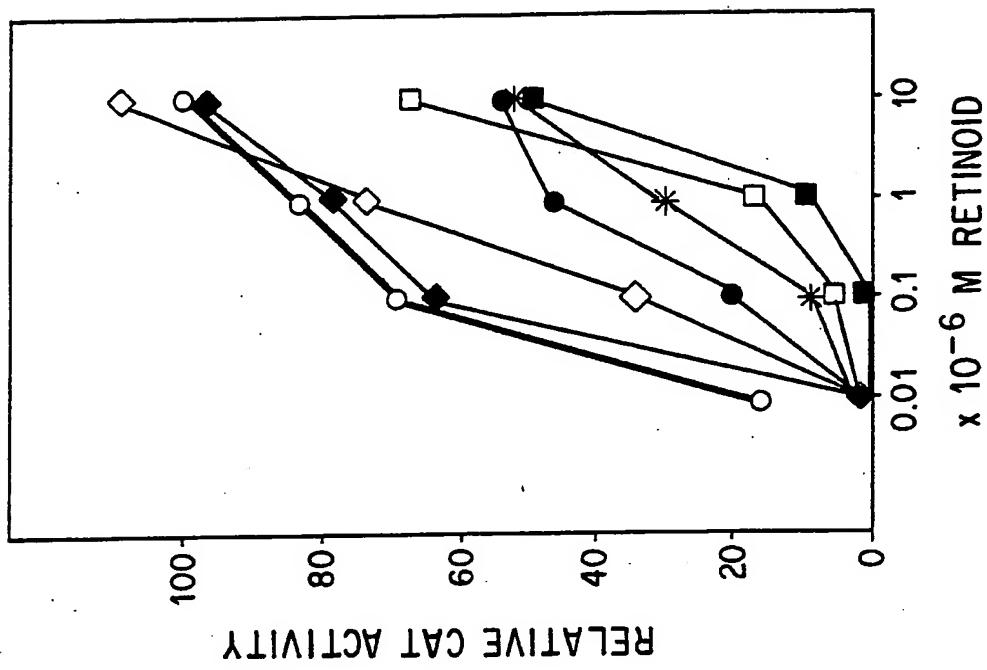


FIG. 7b

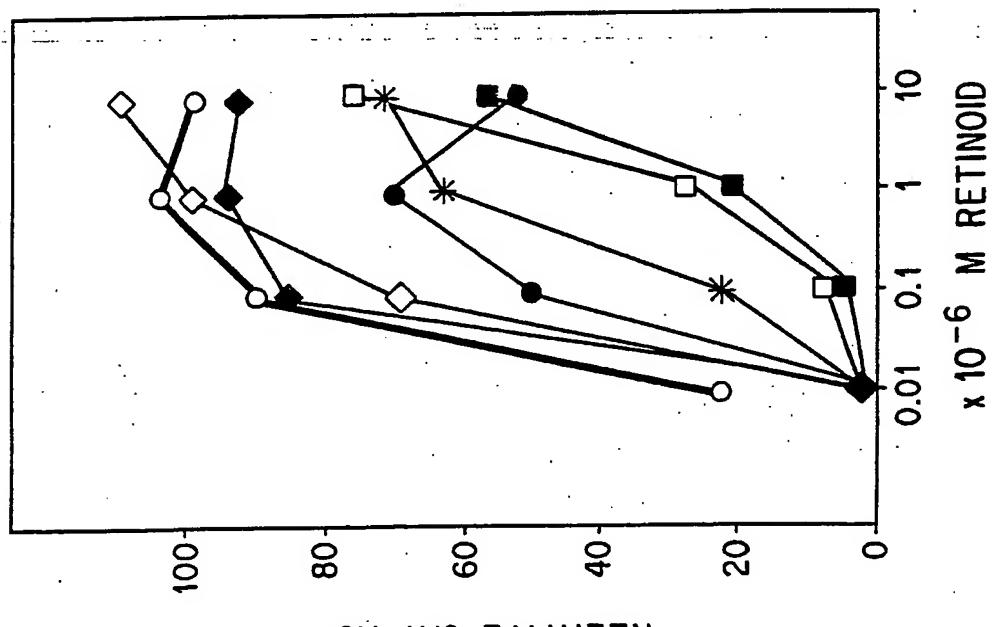


FIG. 7a

RECTIFIED SHEET (RULE 91)  
ISA/EP



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|   |  |   |   |
|---|--|---|---|
| (51) International Patent Classification <sup>5</sup> :<br><br>G01N 33/566, 33/50, C07K 15/06, C07D 339/08, 339/06, 327/04, 319/06, 317/30, 409/04, 333/38, C07C 63/49, 63/66, 65/36, A61K 31/335, 31/19  |  | A3  | (11) International Publication Number: <b>WO 94/12880</b><br><br>(43) International Publication Date: <b>9 June 1994 (09.06.94)</b> |
| (21) International Application Number: <b>PCT/US93/11492</b><br><br>(22) International Filing Date: <b>24 November 1993 (24.11.93)</b>  |  | (74) Agents: PERRYMAN, David, G. et al.; Needle & Rosenberg, 127 Peachtree Street, N.E., Suite 1200, Atlanta, GA 30303 (US).  |   |
| (30) Priority Data:<br>07/982,174 25 November 1992 (25.11.92) US<br>07/982,305 25 November 1992 (25.11.92) US   |  | (81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). |   |
| (71) Applicants: LA JOLLA CANCER RESEARCH FOUNDATION [US/US]; 10901 North Torrey Pines Road, La Jolla, CA 92037 (US). SRI INTERNATIONAL [US/US]; 333 Ravenswood Avenue, Menlo Park, CA 94025 (US).  |  | Published<br><i>With international search report.</i><br><i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>  |   |
| (72) Inventors: PFAHL, Magnus; 605 North Rios Avenue, Solana Beach, CA 92075 (US). ZHANG, Xiao-kun; 9176F Regents Road, La Jolla, CA 92037 (US). LEHMANN, Jürgen, M.; 773 S. Nardo, Apartment H-1, Solana Beach, CA 92075 (US). DAWSON, Marcia, L; P.O. Box 1033, Menlo Park, CA 94026 (US). CAMERION, James, F.; 881 Linda Vista, P.O. Box 69, Moss Beach, CA 94038 (US). HOBBS, Peter, D.; 2176 Williams Street, Palo Alto, CA 94306 (US). JONG, Ling; 1166 Sunnyvale-Saratoga Road, Apartment #22, Sunnyvale, CA 94087 (US). |  | (88) Date of publication of the international search report: <b>29 September 1994 (29.09.94)</b>  |   |
| (54) Title: RXR HOMODIMER FORMATION AND BRIDGED BICYCLIC AROMATIC COMPOUNDS AND THEIR USE IN MODULATING GENE EXPRESSION   |  |   |   |
| <p>RECEPTOR: — RXR      RXR+TR      RXR+RAR      RAR</p> <p>LIGAND: — 9-cis RA T3 — 9-cis T3 9-cis T3+9-cis — 9-cis RA RA+9-cis 9-cis</p> <p>Ab: — — — αF NI<br/>9-cis: — + + +<br/>F-RXR: — + + + +</p>  |  |   |   |

(57) Abstract

The invention provides a method of screening a substance for the ability to affect the formation of a retinoid X receptor homodimer comprising combining the substance and a solution containing retinoid X receptors and determining the presence of homodimer formation. The screening method can be used to determine compounds which selectively activate homodimer formation and heterodimer formation. Also provided is a method of screening a substance for an effect on a retinoid X receptor homodimer's ability to bind DNA comprising combining the substance with the homodimer and determining the effect of the compound on the homodimer's ability to bind DNA. Finally, the invention provides methods of activating retinoid X receptor homodimer formation. Bridged bicyclic aromatic compounds are provided having structure (I); wherein R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and n are as defined herein. The compounds are useful for modulating gene expression of retinoic acid receptors, vitamin D receptors and thyroid receptors. Pharmaceutical compositions and methods for modulating gene expression are provided as well.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

|    |                          |    |                                       |    |                          |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria                  | GB | United Kingdom                        | MR | Mauritania               |
| AU | Australia                | GE | Georgia                               | MW | Malawi                   |
| BB | Barbados                 | GN | Guinea                                | NE | Niger                    |
| BE | Belgium                  | GR | Greece                                | NL | Netherlands              |
| BF | Burkina Faso             | HU | Hungary                               | NO | Norway                   |
| BG | Bulgaria                 | IE | Ireland                               | NZ | New Zealand              |
| BJ | Benin                    | IT | Italy                                 | PL | Poland                   |
| BR | Brazil                   | JP | Japan                                 | PT | Portugal                 |
| BY | Belarus                  | KE | Kenya                                 | RO | Romania                  |
| CA | Canada                   | KG | Kyrgyzstan                            | RU | Russian Federation       |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan                    |
| CG | Congo                    | KR | Republic of Korea                     | SE | Sweden                   |
| CH | Switzerland              | KZ | Kazakhstan                            | SI | Slovenia                 |
| CI | Côte d'Ivoire            | LI | Liechtenstein                         | SK | Slovakia                 |
| CM | Cameroon                 | LK | Sri Lanka                             | SN | Senegal                  |
| CN | China                    | LU | Luxembourg                            | TD | Chad                     |
| CS | Czechoslovakia           | LV | Latvia                                | TG | Togo                     |
| CZ | Czech Republic           | MC | Monaco                                | TJ | Tajikistan               |
| DE | Germany                  | MD | Republic of Moldova                   | TT | Trinidad and Tobago      |
| DK | Denmark                  | MG | Madagascar                            | UA | Ukraine                  |
| ES | Spain                    | ML | Mali                                  | US | United States of America |
| FI | Finland                  | MN | Mongolia                              | UZ | Uzbekistan               |
| FR | France                   |    |                                       | VN | Viet Nam                 |
| GA | Gabon                    |    |                                       |    |                          |

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US 93/11492

| A. CLASSIFICATION OF SUBJECT MATTER |            |            |            |            |            |
|-------------------------------------|------------|------------|------------|------------|------------|
| IPC 5                               | G01N33/566 | G01N33/50  | C07K15/06  | C07D339/08 | C07D339/06 |
|                                     | C07D327/04 | C07D319/06 | C07D317/30 | C07D409/04 | C07D333/38 |
|                                     | C07C63/49  | C07C63/66  | C07C65/36  | A61K31/335 | A61K31/19  |

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 5 C07K G01N A61K C07C C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.       |
|----------|--|-----------------------------|
| X        | NATURE,<br>vol.358, 13 August 1992, LONDON GB<br>pages 587 - 591<br>XIAO-KUN ZHANG ET AL. 'Homodimer formation<br>of retinoid X receptor induced by 9-cis<br>retinoic acid'<br>see the whole document<br>--- | 1-4, 6, 7,<br>12            |
| A        | EP,A,0 260 162 (CENTRE INTERNATIONAL DE<br>RECHERCHES DERMATOLOGIQUES C.I.R.D.) 16<br>March 1988<br>see claims 1-15, 24-31<br>---  | 13-42,<br>65, 66,<br>69, 70 |
|          |  | -/-                         |

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- \*&\* document member of the same patent family

3

Date of the actual completion of the international search

Date of mailing of the international search report

24-08-1994

20 April 1994

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl  
Fax (+ 31-70) 340-3016

Authorized officer

MONTERO LOPEZ B.

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US 93/11492

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.  |
|----------|---|--|
| P,X      | SCIENCE,<br>vol.258, 18 December 1992<br>pages 1944 - 1946<br>JURGEN M. LEHMANN ET AL. 'Retinoids<br>selective for retinoid X receptor response<br>pathways'<br><br>see the whole document<br>--- | 1-4, 6, 7,<br>12-17,<br>19, 20,<br>25-28,<br>32-34,<br>37, 43,<br>46, 47 |
| E        | WO,A,93 25223 (LA JOLLA CANCER RESEARCH<br>FOUNDATION) 23 December 1993<br><br>-----  | 1-7, 12,<br>43, 44,<br>46, 49,<br>50, 54,<br>57, 58,<br>61, 64           |
| 3        |   |  |

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US 93/ 11492

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
Remark : Although claims 54-60, 67-70 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2.  Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see annex

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-7, 12-50, 54-70

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

- 1.- claims 1-7,12-50,54-70: Method for screening a substance for the ability to affect RXR homodimer formation. Substances thereof and their uses to affect homodimer formation, and compositions containing them. Purified RXR homodimers.
- 2.- claim 8: Method for screening a substance which induces RXR heterodimer formation.
- 3.- claim 9: Method for screening a substance which affects RXR homodimer binding to DNA.
- 4.- claims 10,11,51,52: Method for screening response elements for binding to RXR homodimers.
- 5.- claim 53: Method for detecting a pathology associated with RXR homodimer formation.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 93/11492

| Patent document cited in search report | Publication date | Patent family member(s) |          | Publication date |
|--|------------------|-------------------------|----------|------------------|
| EP-A-0260162                           | 16-03-88         | FR-A-                   | 2601670  | 22-01-88         |
|  |                  | AU-B-                   | 597396   | 31-05-90         |
|  |                  | AU-A-                   | 7590387  | 04-02-88         |
|  |                  | CA-A-                   | 1328605  | 19-04-94         |
|  |                  | ES-T-                   | 2002463  | 16-01-94         |
|  |                  | JP-A-                   | 63030433 | 09-02-88         |
|  |                  | US-A-                   | 4833240  | 23-05-89         |
| -----                                  | -----            | -----                   | -----    | -----            |
| WO-A-9325223                           | 23-12-93         | AU-B-                   | 4636893  | 04-01-94         |
| -----                                  | -----            | -----                   | -----    | -----            |

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER: \_\_\_\_\_**

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**